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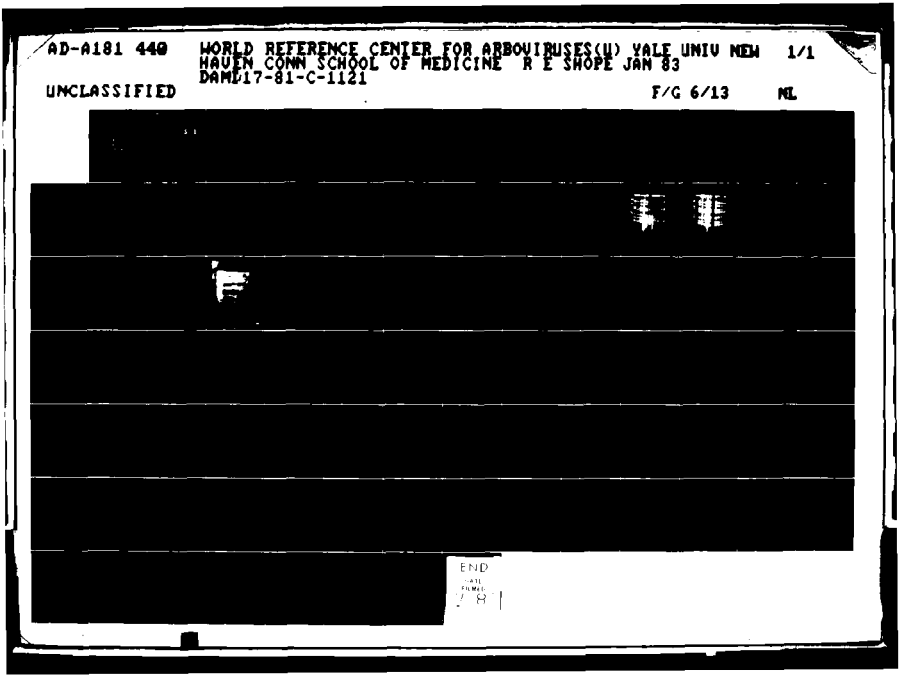

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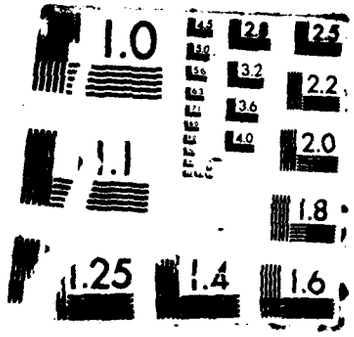
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WORLD REFERENCE CENTER FOR ARBOVIRUSES

ANNUAL PROGRESS REPORT

Robert E. Shope, M.D.

January 1983

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Revisions were made of the genera Phlebovirus, Orbivirus, Bunyavirus, and the family Rhabdoviridae. New viruses of the Kemerovo and Sakhalin serogroups were identified. Serological evidence of phlebovirus antibody was found in sera from Greece, and of flavi- alpha- and bunyaviruses in sera from the Sudan. Zinga virus was identified as Rift Valley fever virus, extending the range from Senegal to Madagasgar. Rift Valley fever antibody was shown to persist 49 years after infection; the antibody was still in high titer. A new technique to conduct hybridization of ssRNA was developed as a modificatio

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of the Southern blot. Monoclonal antibody to Rift Valley fever virus established that there are at least 6 surface-reactive sites, 4 of these involved in the neutralization reaction. The ELISA was used effectively to screen Rift Valley fever vaccinees for antibody. Both the IgM and IgG ELISA were developed for Rift Valley fever. The ELISA was also adapted to Crimean-Congo hemorrhagic fever virus for antibody detection. Antigen-detection ELISA was applied to dengue, and in another study to eastern encephalitis and Highland J viruses for detection in mosquito pools, both infected in the laboratory and infected in nature. The correlation with conventional virus isolation methods was excellent. A collection of low passage arboviruses was continued. Fifty-two new acquisitions, including Japanese encephalitis, dengue, yellow fever, phlebotomus fever viruses, and Ross River, were lyophilized and are available for investigators on request. The reference center distributed 568 ampoules of reference sera, antigens, and viruses during 1982. Of the viruses distributed, there were represented 110 different serotypes.

Keywords: viruses; hemorrhagic fever viruses.

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SUMMARY

Virus classification. Taxonomic study of phleboviruses has led to the discovery that Zinga virus, previously believed to be ungrouped, is a strain of Rift Valley fever (RVF) virus. This surprising finding extends the known range of RVF from Senegal to Madagascar. A New World phlebovirus, Belterra, was closely related to RVF by CF and neutralization tests. Identification of a new phlebovirus from Italy brings the number of known serotypes to 36. Three new rhabdoviruses from Australia were distantly related to each other and two of them to VSV-New Jersey. The Tete group of bunyaviruses was revised to include a new bird isolate from India. Two major serogroups of orbiviruses were studied by serological tests and by PAGE. The genetic variance in the Changuinola group was amazingly broad with at least 20 distinct profiles. That in the Palyam group was quite narrow. This suggests that Palyam group viruses are genetically quite stable.

Virus identification. New viruses were identified in the Kemerovo group from Alaska and France, and in the Sakhalin group from France. For the first time, the Reference Center is receiving viruses isolated in mosquito cells; some of these appear to be mosquito viruses but some others appear to be arboviruses which do not infect laboratory animals. New methods are being developed to replace classical techniques for identifying these agents.

Serologic survey. Serosurveys were undertaken in Egypt and Sudan where 20% flavi- and a lower percentage of alpha- and bunyavirus antibodies were found. RVF IFA, ELISA, and neutralizing antibody was detected in high titer in sera of a laboratory technician infected in New York 49 years ago. Human sera from Greece, some of which have RVF HI reactivity, have a high prevalence of Naples sandfly fever virus neutralizing antibody which may account in part for the RVF cross-reactivity.

Diagnosis of disease. Dengue was diagnosed in a traveller returning to Connecticut from Puerto Rico, but not in accompanying persons who had similar illness. Dengue neutralizing antibody rises were recorded in 6% of the CNS disease cases in a pediatric hospital in Jakarta; these children had no antibody rises to JE virus. Six of 25 U.S. Navy personnel who reported fever during the 1981 dengue-2 outbreak had dengue-2 neutralizing antibody. Almost none of those not reporting ill developed antibody. A Canadian developing encephalitis on returning from Manchuria had a postive IgM antibody capture ELISA with JE antigen. An outbreak of fever in Spaniards working in Libya was negative with arbovirus antigens and remains undiagnosed.

Development of techniques. Major emphasis was placed on monoclonal antibodies and ELISA as methods for early and rapid diagnosis and identification of viruses. IgG and IgM ELISA functioned well with Rift Valley fever. The ELISA correlated with PRNT tests of post-vaccination human sera. The ELISA was a broadly cross-reacting test with both human and mouse immune sera immune to other phleboviruses. An 8-minute latex agglutination test was adapted from rubella to arboviruses. It was more

rapid and more sensitive than the HI test. The Crimean hemorrhagic fever-Congo virus ELISA was also employed and the technology transferred to a laboratory in Greece. Monoclonal antibodies for Rift Valley fever were generally specific, all IgG, reacted the same in HI and neutralization when directed to glycoproteins and in CF when directed to nucleocapsids. There were 6 antigenic sites, 4 of them involved in neutralization.

The antigen-detection ELISA was used to identify infected pools of mosquitoes. With eastern encephalitis and Highland J viruses, the correlation was excellent with results of virus isolation using conventional techniques and field specimens from Massachusetts.

A series of experiments with yellow fever virus and mosquitoes failed to demonstrate transmission by nectar, failed to show any marked effects of colonization through 6 generations on susceptibility to infection, and failed to show a difference between fresh and frozen meals in capacity to infect mosquitoes.

A modification of the Southern blotting technique was applied successfully to hybridize ssRNA of orbiviruses after the dsRNA was denatured by glyoxalation. This novel technique permits identification and comparison of RNA viruses at the genome level and should be applicable also to ssRNA viruses.

Collection of low passage virus reference strains. A collection of 52 unpassaged or low passage arboviruses was made, aliquoted, and lyophilized to be sent to interested scientists and to be stored for future experimental approaches. These include Highland J, eastern encephalitis, phlebotomus fever viruses, dengue viruses, Japanese encephalitis, and several other flaviviruses, rhabdoviruses, and bunyaviruses.

Distribution of reagents. The reference centre distributed 568 ampoules of reference sera, antigens, and viruses during 1982; mosquito, tick, and vertebrate cells were also distributed. Of the viruses distributed, there were represented 110 different serotypes.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences - National Research Council.

BODY OF REPORT

I. VIRUS CLASSIFICATION

PHLEBOVIRUSES

Use of Monoclonal Antibodies to Identify Zinga Virus as Rift Valley Fever Virus (J. Meegan, J.P. Digoutte, C.J. Peters, R.E. Shope). A significant cross-reaction was detected between Zinga virus (ZV) and RVF virus (RVFV) while testing antisera to a number of African viruses in an indirect fluorescent antibody (IFA) test using inactivated RVFV infected cells as antigen. Until now, ZV was considered a serologically ungrouped arthropod-borne virus isolated from man and mosquitoes in central and western Africa. Because of the biohazard RVFV represents to man and animals, research on it is restricted to high containment laboratories. Consequently, it was desirable to determine rapidly if ZV was RVFV or a related virus in the phlebotomus fever group of the family Bunyaviridae.

We recently characterized a bank of monoclonal antibodies developed against inactivated RVF virion antigens (see V. Development of new techniques). Three of these were selected for testing ZV. Each reacts in the IFA test with all strains of RVFV, and binds to non-overlapping antigenic sites present on three different virion structural proteins. Two of these monoclonal antibody preparations bind to sites on the two surface glycoproteins and show no cross-reactions when tested against the 33 other viruses of the phlebotomus fever serogroup. Additionally, one of these monoclonal preparations efficiently inhibits hemagglutination and neutralizes the virus. The third monoclonal antibody binds to a site on the nucleocapsid protein, and cross-reacts in the IFA test with four phleboviruses, all isolated only from South America. It was postulated that if these three monoclonal antibodies reacted to high titer with ZV, it would be a strain of RVFV.

Table 1 shows IFA results indicating that each monoclonal antibody reacted to titer with RVFV and ZV infected cells. Based on these results, ZV was sent to a high containment laboratory (USAMRIID) where virus stocks were prepared, titered, and plaque reduction neutralization (PRNT) tests were performed. Table 2 reveals PRNT results indicating ZV is closely related to the Zagazig 501 and Entebbe strains of RVFV. The differences in titer are reproducible and could indicate slight antigenic differences which should be further investigated. However, since none of the other phlebotomus fever group viruses have shown such significant PRNT cross-reactions with RVFV, these differences between ZV and RVFV should be considered minor strain variations. Preliminary animal inoculation studies indicate ZV is a classic pantropic, virulent strain of RVFV.

In this study, the use of defined monoclonal antibodies in an IFA test provided an advantage over the PRNT test. Since we had produced and inactivated ZV infected cells as antigens, and had been supplied with inactivated RVFV antigen slides, the IFA test was easily and rapidly performed with a minimal biohazard. An IFA test using reference polyclonal antisera to RVFV would have cross-reacted with other phleboviruses. For laboratories where reference virus strains cannot be grown and titered for PRNT tests, or for laboratories attempting to identify new isolates as one of the over 500 arthropod-borne viruses, specific monoclonal antibodies, and more importantly those that are broadly cross-reactive within a group, represent valuable new reagents.

ZV has been isolated from mosquitoes collected in Central African Republic (CAR), Senegal, Madagascar, and Guinea, and from naturally infected humans in

Senegal and CAR. The isolations extend the confirmed range of RVFV circulation into West Africa, and add two new mosquitoes, Aedes (Aedimorphus) dalzieli and Ae. (Neomelaniconior) palpalis group to the list of over 20 mosquitoes implicated as possible vectors of RVFV in Africa.

Zinga strain of RVFV is similar to other African RVFV strains in its pathogenicity in laboratory animals, and apparently in its ability to infect humans. However, there are no reports of epizootics caused by ZV and no isolations of ZV from animals. Although local outbreaks may have been misdiagnosed, he repeated individual isolations of ZV during periods when active field studies were ongoing in CAR and Senegal suggest RVFV has recently circulated in a non-epizootic form in these areas.

During the last few years ZV has been studied in many laboratories in Nort America, Europe, and Africa. Numerous ZV infections have resulted from laboratory accidents. Any laboratory with ZV in its collection should be aware it is a strain of RVFV and represents a substantial biohazard to people and animals.

Table 1 - Indirect fluorescent antibody assay of ZV using monoclonal antibodies to RVFV

	Antigen		
Antisera	ZV	RVFV	Other**
Monoclonal 4328D	≥10000	≥10000	≤10
Monoclonal 1295F	≥10000	≥10000	≤10
Monoclonal 143P	≥10000	≥10000	≤10
Polyvalent anti-RVFV	160	160	≤10-80

*Reciprocal of highest dilution giving positive results. Antigen preparation and test methodology as described in Tesh RB, Peters CJ, Meegan JM. Studies the antigenic relationship among phleboviruses. Am J Trop Med Hyg 31:149-15 1982.

**Other phleboviruses from Africa: sandfly fever Sicilian, sandfly fever Naple Gordil, Saint-Flores, Arumowot, and Gabek Forest.

Table 2 - Plaque reduction neutralization tests of RVFV and ZV

Antibody	Virus		
	ZV	RVFV-Zagazig 501	RVFV-Entebbe
Anti-ZV	160*	320	40
Anti-RVFV-Zagzig 501	2560	10240	10240
Anti-RVFV-Entebbe	160	1280	1280

*100 plaque forming units of RVFV incubated for 1 hr at 37°C with antibody before assay for residual infectivity in Vero cells to determine highest dilution neutralizing 80% of inoculum.

Antigenic relationship among phleboviruses (R.B. Tesh, A. Travassos, J. Travassos and J.M. Meegan). In 1981, complement fixation (CF) tests were done comparing 34 known phlebovirus antigens and antisera. These results were reported in the 1981 Annual Report. During the current year, additional CF tests were done to include 2 other phleboviruses (Rift Valley fever and ISS.Phl.18). A complete summary of the CF results is shown in Table 3. From these data, it appears that there are at least 6 antigenic complexes within the phlebotomus fever serogroup. Furthermore, by CF test some of the virus serotypes are practically indistinguishable. Perh ps the most interesting finding in this serologic study was the close antigenic relationship demonstrated between Rift Valley fever, Belterra and Icoaraci viruses (Table 4).

Because of the close antigenic relationship among some phleboviruses by CF test, plaque reduction neutralization tests (PRNT) were done on selected members of the group. These results are shown in Tables 5 and 6. Neutralization tests with Rift Valley fever virus were done by Dr. C. J. Peters, USAMRIID, Fort Detrick. By PRNT, each of the viruses could be differentiated. Interestingly, the antigenic relationship among Rift Valley fever, Belterra and Icoaraci viruses was confirmed by PRNT. In fact, the Belterra mouse hyperimmune ascitic fluid inhibited Rift Valley fever virus as well as it did the homologous virus.

Results of these studies increase the known serotypes in the genus Phlebovirus (phlebotomus fever serogroup) to 36.

Table 3

Results of complement fixation tests with 36 phlebotomus fever group virus serotypes

ANTIGEN	ANTI SERUM																																	
	ACO	BOJ	MON	RVF	STA	IGO	CHG	GOR	BG	ITA	ALE	PIQ	CDG	TOA	DRE	PT	DUE	TOR	TEN	SPH	KAR	VNU	PAC	ITP	SFS	AVN	PBI	JOA	CAC	CHI	CAT	SAP	ANT	CP	SAL
Ag-acoto	2560/128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bajaru	0/32	256/128	256/128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nungubo	16/128	128/0	256/128	0	0	0	0/0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/0	0	0	0	0	0	0	0	0	0
Rift Valley fever	0	0	0/16	128/256	256/256	32/64	0/16	0/0	0/0	32/32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Belterra	0	0	0	0	256/128	16/128	0	0	0	0	0	0	0	0	0	32/32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Icoaraci	0	0	0	-	256/128	128/128	0	0	0	0	0	0	0	0	0	0	0	0/1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chagres	0	0	0	-	0	0	256/128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cordil	0	0	0	-	0	0	0	256/32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rio Grande	0	0	0	-	0	0	0	0	256/0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Itaituba	0	0	0	-	0	0	0	0	0	256/128	0	16/128	256/128	128/32	64/32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Almeguer	0	0	0	-	0	0	0	0	0	0	256/32	256/0	0	64/0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Uiqua	0	0	0	-	0	0	0	0	0	32/0	64/0	256/0	0/0	256/0	16/0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Candiru	0	0	0	-	0	0	0	0	0	256/128	0	16/128	256/128	64/128	64/128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Taruma	0	0	0	-	0	0	0	0	0	64/32	32/32	128/32	64/32	256/32	8/0	16/0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oriximina	0	0	0	0	0	0	0	0	0	128/128	16/128	128/128	256/128	64/128	256/128	16/32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ponta Pora	0	0	0	-	0	0	0	0	0	0	0	32/128	0	16/128	0	256/128	16/128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pontaventura	0	0	0	-	0	0	0	0	0	0	0	0	0	8/32	0	128/128	256/128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tacaiuma	0	0	0	-	16/0	0	0	0	0	0	0	0	0	0	0	0	0	256/128	256/128	64/128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Tehrun	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	128/32	256/128	64/128	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Naples	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	256/32	256/32	256/32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carimbod	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	32/0	128/0	32/0	32/128	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cruzeri	0	0	0	-	0/0	0	0	0	0	0	0	0	0/0	0	0	0	0	0/0	0	0	0	64/32	0	0	0	0	0	0	0	0	0	0	0	0	0
Pacui	0	0	0	-	0/32	0	0	0	0	0	0	0	0	0	0	0	0	16/0	0	0	0	0	256/32	0	0	0	0	0	0	0	0	0	0	0	0
Itaporanga	0	0	0	-	0/0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256/0	0	0	0	0	0	0	0	0	0	0	0
Sicilian	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<8	0	256/32	0	0	0	0	0	0	0	0	0	0
Ambooga	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256/0	0	0	0	0	0	0	0	0	0
Frijoles	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256/32	32/0	0	0	0	0	0	0	0	0
Ana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256/128	32/32	0	0	0	0	0	0	0	0
Cocao	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256/128	256/32	0	0	0	0	0	0
Chilibre	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256/128	0	0	0	0	0	0
Caimito	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256/0	0	0	0	0	0
Saint Florie	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256/32	0	0	0	0
Arumanet	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256/128	0	0	0
Ashah Forest	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256/128	0	0
Sulahabad	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	256/128	0
PHS.Pbf. 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	256/128	0

* Reciprocal of highest antiserum dilution/highest antigen dilution.
0 = < 1/8 - = indicates not tested

Table 4

Results of CF tests

	Antiserum*		
Antigen	RVF	Belterra	Icoaraci
RVF	>256/>256*	>256/256	32/64
Belterra	<4/4**	>256/>128	16/>128
Icoaraci	<4/4	256/128	128/128

*Reciprocal of highest antiserum dilution/highest antigen dilution.

**Not tested.

The RVF antigen used was a BPL-treated infected mouse liver. The immune serum was a rabbit antiserum received from USAMRIID.

Table 5

Results of plaque reduction neutralization tests with selected phlebotomus fever group viruses.

	ANTISERUM										
VIRUS	BUJ	MUN	RVF	BTA	ICO	ITA	ALE	NIQ	CDU	TUA	ORX
BUJURU	1,280*	160	-	-	-	-	-	-	-	-	-
MUNGUBA	80	5,120	-	-	-	-	-	-	-	-	-
RIFT VALLEY FEVER	<10	<10	320	640	40	<10	<10	<10	-	<10	<10
BELTERRA	-	-	<10	320	2,560	-	-	-	-	-	-
ICOARACI	-	-	<10	<10	10,240	-	-	-	-	-	-
ITAITUBA	-	-	<10	10	<10	5,120	<10	640	640	<10	320
ALENQUER	-	-	-	-	-	<10	80	10	10	10	<10
NIQUE	-	-	-	-	-	<10	<10	80	40	20	<10
CANDIRU	-	-	-	-	-	<10	<10	<10	1,280	10	<10
TURUNA	-	-	-	-	-	<10	<10	40	320	1,280	40
ORIXIMINA	-	-	-	-	-	160	20	160	320	20	160

* Reciprocal of highest antiserum dilution producing 90% plaque inhibition.
- not tested

Table 6

Results of plaque reduction neutralization tests with selected phlebotomus fever group viruses

	ANTISERUM								
VIRUS	PT	BUE	TOS	TEH	SFN	FRI	JOA	SAL	ISS
PUNTA TORO	1,280*	40	-	-	-	-	-	-	-
BUENAVENTURA	20	40	-	-	-	-	-	-	-
TOSCANA	-	-	>5,120	<10	40	-	-	-	-
TEHRAN	-	-	<10	80	<10	-	-	-	-
NAPLES	-	-	20	<10	320	-	-	-	-
FRIJOLES	-	-	-	-	-	5,120	1,280	-	-
JOA	-	-	-	-	-	320	10,240	-	-
SALEHABAD	-	-	-	-	-	-	-	320	<10
ISS.Ph1.18	-	-	-	-	-	-	-	<10	160

* Reciprocal of highest antiserum dilution producing 90% plaque inhibition.
\- not tested.

RHABDOVIRUSES

Serologic studies with 3 new Australian rhabdoviruses (R.B. Tesh & A.J. Main). In the 1981 Annual Report, results of serological studies with 51 known vertebrate rhabdoviruses were described. During 1982, we received 3 new mosquito-associated rhabdoviruses (Kimberly, Kununurra and Parry Creek) from Dr. N. Stanley, Perth, Australia. Kimberly, Kununurra and Parry Creek antigens were examined by CF test against the following antigens and/or mouse immune ascitic fluids: Kimberly, Kununurra, Parry Creek, Kamese, Charleville, Barur, Jurona, Obodhiang, Aruac, Inhangapi, Porton S, Tibrogargan, VSV-New Jersey, VSV-Indiana, rabies, Lagos bat, Mokola, Duvenhage, Kotonkan, Joinjakaka, Mt Elgon bat, Yata, Kwatta, Timbo, Almpiwar, DakA94, Kwatta, and bovine ephemeral fever. By CF test, Kimberly, Kununurra and Parry Creek were interrelated as shown in Table 7. The antigenic relationship between Kimberly, Kununurra and Parry Creek was also confirmed by indirect fluorescent antibody test. However, by plaque reduction neutralization test, these 3 agents were distinct (Table 8). Interestingly, Kununurra antiserum also reacted at a 1:4 dilution with VSV-New Jersey antigen, and VSV-New Jersey antiserum reacted at the same dilution with Kimberly antigen in cross-CF tests. These latter reactions will be rechecked in the near future.

Table 7

Results of complement fixation tests with Kimberly, Kununurra and Parry Creek viruses

	ANTISERUM		
VIRUS	Kimberly	Kununurra	Parry Creek
Kimberly	512/≥32*	4/4	4/4
Kununurra	8/16	32/16	<4/4
Parry Creek	8/16	8/8	128/≥32

*Reciprocal of highest positive antiserum dilution/highest positive antigen dilution.

Table 8

Results of plaque reduction neutralization tests with Kimberly, Kununurra and Parry Creek viruses

	ANTISERUM		
	Kimberly	Kununurra	Parry Creek
VIRUS			
Kimberly	1,280*	<10	<10
Kununurra	<10	160	<10
Parry Creek	<10	<10	2,560

*Reciprocal of highest antiserum dilution producing>90% plaque inhibition.

ORBIVIRUSES

Characterization of the Changuinola virus serogroup (R.B. Tesh and D.L. Knudson). The Changuinola serogroup consists of a large number of antigenically related viruses which have been associated with phlebotomine sandflies, mosquitoes, and various species of wild mammals. These viruses have been found only in Tropical America, and they are presumed to be arthropod-borne. On the basis of their physicochemical and morphological properties, they are included in the family Reoviridae, genus Orbivirus.

Between 1960 and 1980, a total of 178 Changuinola group virus strains were isolated from various geographic localities in Brazil, Colombia and Panama. Samples of each of these virus strains have been collected at YARU and are available for study. We are presently attempting to characterize the Changuinola serogroup by comparing the biological, serological and chemical properties of some of its members. A sample of 24 representative Changuinola group viruses were selected for study. These agents are listed in Table 9. Last year, we reported results of complement-fixation (CF) and neutralization tests done with some of these viruses. By CF test, the viruses were indistinguishable; but by neutralization technique, at least 12 distinct serotypes were identified. During 1982, polyacrylamide gel electrophoresis of ds RNA of the 24 viral agents was done. By this technique, 20 distinct profiles were identified. These profiles are shown in Figures 1 and 2. Viruses which were indistinguishable by neutralization test had identical RNA profiles. These data indicate that the Changuinola group is probably comprised of a large number of different virus serotypes. A manuscript is now in preparation describing this heretofore obscure arbovirus serogroup.

Growth of the prototype Changuinola virus strain (BT-436) was compared in mosquito and sandfly cell cultures. Insect cell cultures used were the C6/36 clone of Aedes albopictus and a newly established cell line (LL-5) from the sandfly, Lutzomyia longipalpis. Each cell line was inoculated with 100 PFU of BT-436 virus. Samples of the infected cells were taken daily, frozen, and subsequently titrated in monolayer cultures of Vero cells. The results are shown in Table 10. BT-436 virus grew equally well in both cell lines. Interestingly, BT-436 virus produced transient cytopathic effect in the LL-5 cells; but after the cells regenerated a persistent infection was established.

Table 9

Changuinola serogroup viruses included in this study

Virus*	Strain number	Source	Geographic locality**	Date of isolation
Changuinola	BT-436	Lutzomyia sp. (sandfly)	Bocas del Toro*, Panama	1960
-	BT-104	Lutzomyia sp.	Bocas del Toro, Panama	1960
-	BT-766	Lutzomyia sp.	Bocas del Toro, Panama	1960
-	BT-2164	Lutzomyia sp.	Bocas del Toro, Panama	1961
-	BT-2365	Lutzomyia sp.	Bocas del Toro, Panama	1961
-	BT-2380	Lutzomyia sp.	Bocas del Toro, Panama	1961
-	VP-19A	Lutzomyia sp.	Panama, Panama	1969
-	VP-46F	Lutzomyia sp.	Panama, Panama	1969
	VP-188G	Lutzomyia trapidoi	Panama, Panama	1970
-	VP-202A	Lutzomyia sp.	Panama, Panama	1970
-	CoAr 2837	Lutzomyia sp.	Valle, Colombia	1964
Irituia	BeAn 28873	Oryzomys sp. (rice rat)	Para, Brazil	1961
Gurupi	BeAr 35646	Lutzomyia sp.	Para, Brazil	1962
Ourem	BeAr 41067	Lutzomyia sp.	Para, Brazil	1962
Caninde	BeAr 54342	Lutzomyia sp.	Para, Brazil	1963
Jamanxi	BeAr 243090	Lutzomyia sp.	Para, Brazil	1973
Altamira	BeAr 264277	Lutzomyia sp.	Para, Brazil	1974
Purus	BeAr 361064	Psorophora albipes (mosquito)	Acre, Brazil	1977
Jari	BeAn 385199	Choloepus didactylus (sloth)	Para, Brazil	1980
Saraca	BeAr 385278	Lutzomyia sp.	Para, Brazil	1980
Monte Dourado	BeAn 385401	Dasypus novemcinctus (armadillo)	Para, Brazil	1980
Almeirim	BeAr 389709	Lutzomyia umbratilis	Para, Brazil	1980
-	BeAr 385274	Lutzomyia sp.	Para, Brazil	1980
-	BeAr 385279	Lutzomyia sp.	Para, Brazil	980

* Hyphen denotes unnamed strain; ** State province department, country

Figure 1

Changuinola serogroup: DsRNA profiles

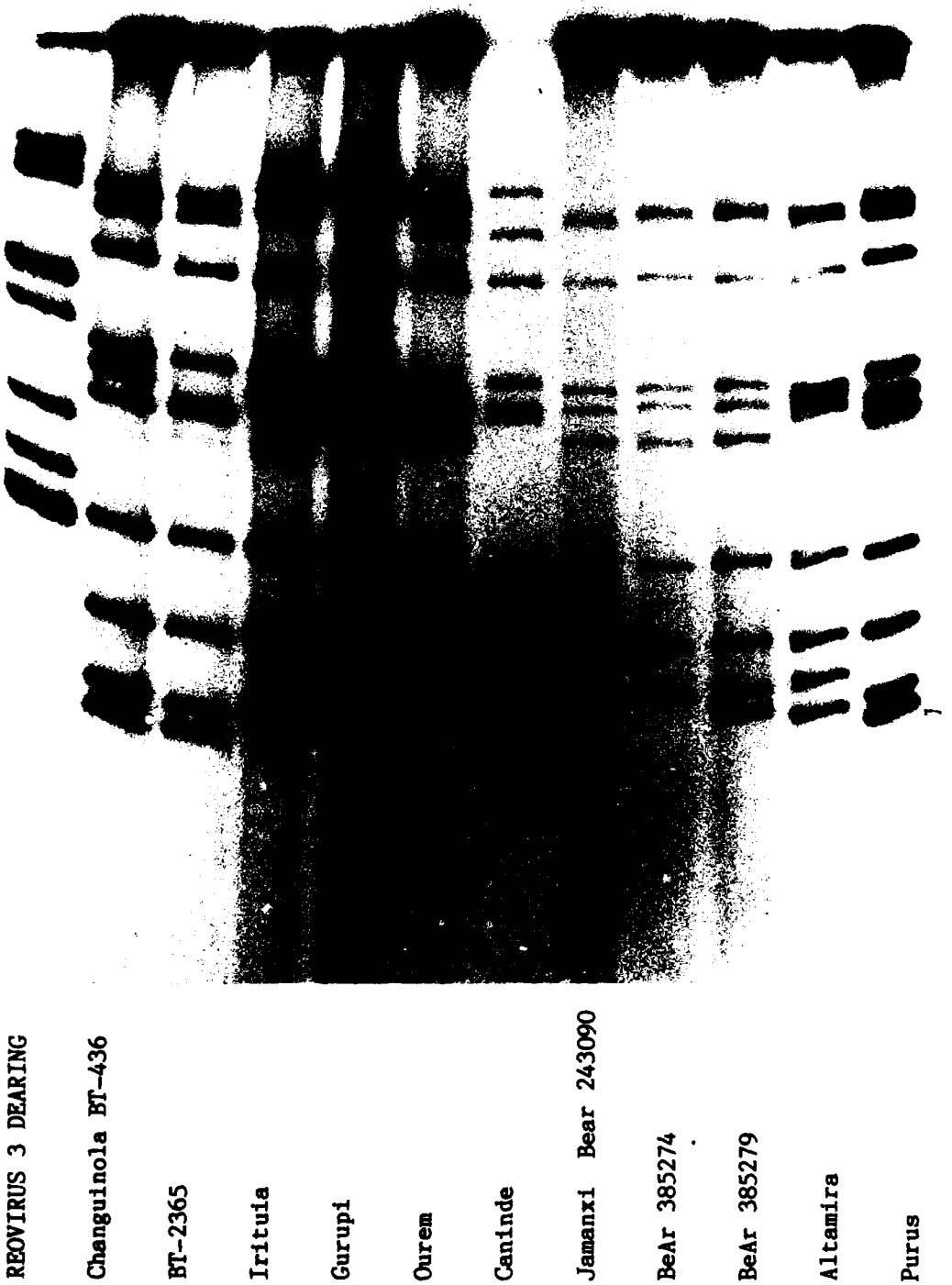


Figure 2

Changuinola serogroup: DsRNA profiles

Table 10

Growth of Changuinola virus (BT-436) in Lutzomyia longipalpis (LL-5) and Aedes albopictus (C6/36) cells

Day post-inoculation	Virus titer* LL-5	C6/36
1	1.0	0.7
2	3.5	3.9
3	5.5	5.2
4	6.0	6.2
5	6.2	6.4
6	5.9	6.6
7	6.4	6.7

*Titer expressed as $\log_{10}$ of PFU/ml of frozen cell harvest.

Characterization of the Palyam Virus Serogroup (R.B. Tesh, D.L. Knudson, A.J. Main). Viruses included in the Palyam serogroup have been associated with a variety of arthropods and large mammals (principally bovines) in Africa, Asia and Australia. On the basis of their physiochemical and morphological properties, these agents are included in the family Reoviridae, genus Orbivirus. Because of their frequent recovery from blood-sucking arthropods (mosquitoes, ticks and Culicoides midges), they are presumed to be vector-borne. In view of their taxonomic and biological similarities with other orbiviruses causing disease in humans and animals (African horsesickness, bluetongue, Colorado tick fever, equine encephalosis, epizootic disease of deer, Kemerovo and Orungo viruses), the Palyam group agents may also be of some veterinary or public health importance. In fact, one member of the Palyam serogroup (Nyabira) has been isolated on several occasions from aborted bovine fetuses in East Africa. However, at present, the disease potential of other viruses in this serogroup is still unknown.

During the past year, 31 Palyam serogroup viruses were examined by complement-fixation (CF) and plaque reduction neutralization (PRN) tests and by polyacrylamide gel electrophoresis (PAGE). Ten distinct virus serotypes were identified. These agents as well as their source, geographical origin, and date of isolation are listed in Table 11. By CF test, these viruses were indistinguishable, as shown in Table 12. However, by PRN test each of the viruses was distinct, except for Abadina and DakArK58 which appeared to be strains of the same virus (Table 13). Similar results were obtainted by PAGE: viruses which were indistinguishable by PRN test had identical RNA profiles, whereas those agents which were distinct by neutralization test also had different RNA patterns (Figure 3). A sample of 23 Palyam group viruses, collected in Australia over a 9-year period (1972-1981), were also examined by PAGE. These agents had previously been identified by neutralization test as D'Aguilar, Bunyip Creek and CSIRO Village viruses. Results of PAGE indicated that those viruses belonging to the same serotype had identical RNA profiles, suggesting that these agents are genetically stable.

Table 11

Palyam group viruses examined by PRNT in this study

Virus identification	Strain	Source	Geographical origin	Date of isolation
Palyam	G5287	Culex "vishnui"	Vellore, India	1956
Kasba	Gl5534	Culex "vishnui"	Vellore, India	1957
Vellore	68886	Culex pseudovishnui	Vellore, India	1966
D'Aguilar	B8112	Culicoides brevitarsis	Bunya, Q., Australia	1972
CSIRO Village	CSIRO 11	Culicoides spp.	Beatrice Hill, N.T., Australia	1974
Bunyip Creek	CSIRO 87	Culicoides schultzei	Beatrice Hill, N.T., Australia	1976
Marrakai	CSIRO 82	Culicoides schultzei + peregrinus	Beatrice Hill, N.T., Australia	1975
Nyabira	792/73	calf (aborted fetus)	Nyabira, Zimbabwe	1973
Abadina	IB Ar 22388	Culicoides spp.	Ibadan, Nigeria	1967
Dak Ar B 2032	Dak Ar B 2032	Amblyomma variegatum	Bangui, Central African Republic	1978
Dak Ar K 58	Dak Ar K 58	Amblyomma variegatum	Kindia, Rep. of Guinea	1978

Table 12

Results of complement fixation tests with Palyam serogroup viruses

ANTIGEN	ANTISERUM										
	PALYAM	CSIRO VILLAGE	MARRA-KAI	BUNYIP CREEK	VELLORE	B2032	KASBA	ABADINA	K58	D'AGUILAR	NYABIRA
PALYAM	128/32*	64/32	128/64	64/32	256/128	256/32	256/64	256/32	64/16	256/64	128/64
CSIRO VILLAGE	16/16	128/8	32/4	64/32	128/64	128/8	128/4	64/8	8/4	256/16	128/8
MARRAKAI	128/64	128/64	256/64	128/64	256/128	128/64	256/64	256/128	64/32	512/64	128/≥128
BUNYIP CREEK	128/32	64/64	128/64	128/128	128/≥128	128/64	256/64	128/64	32/32	256/64	128/≥128
VELLORE	128/64	128/64	128/64	64/64	512/≥128	256/64	256/64	256/64	64/32	256/64	128/≥128
DAK AR B2032	128/64	128/64	128/128	64/128	256/≥128	256/128	256/128	256/128	64/64	256/≥128	128/≥128
KASBA	128/64	128/64	128/64	128/64	256/≥128	256/64	512/128	256/64	64/32	512/64	128/≥128
ABADINA	128/32	64/32	128/64	64/64	256/≥128	128/64	256/64	256/64	64/64	256/64	128/≥128
DAK AR K58	32/16	128/16	128/16	64/32	256/64	128/16	256/16	256/16	64/8	256/16	128/32
D'AGUILAR	128/64	64/64	128/64	128/64	128/≥128	128/64	256/64	256/64	32/32	512/≥128	128/≥64

* Reciprocal of highest antiserum dilution/ highest antigen dilution.

Table 13

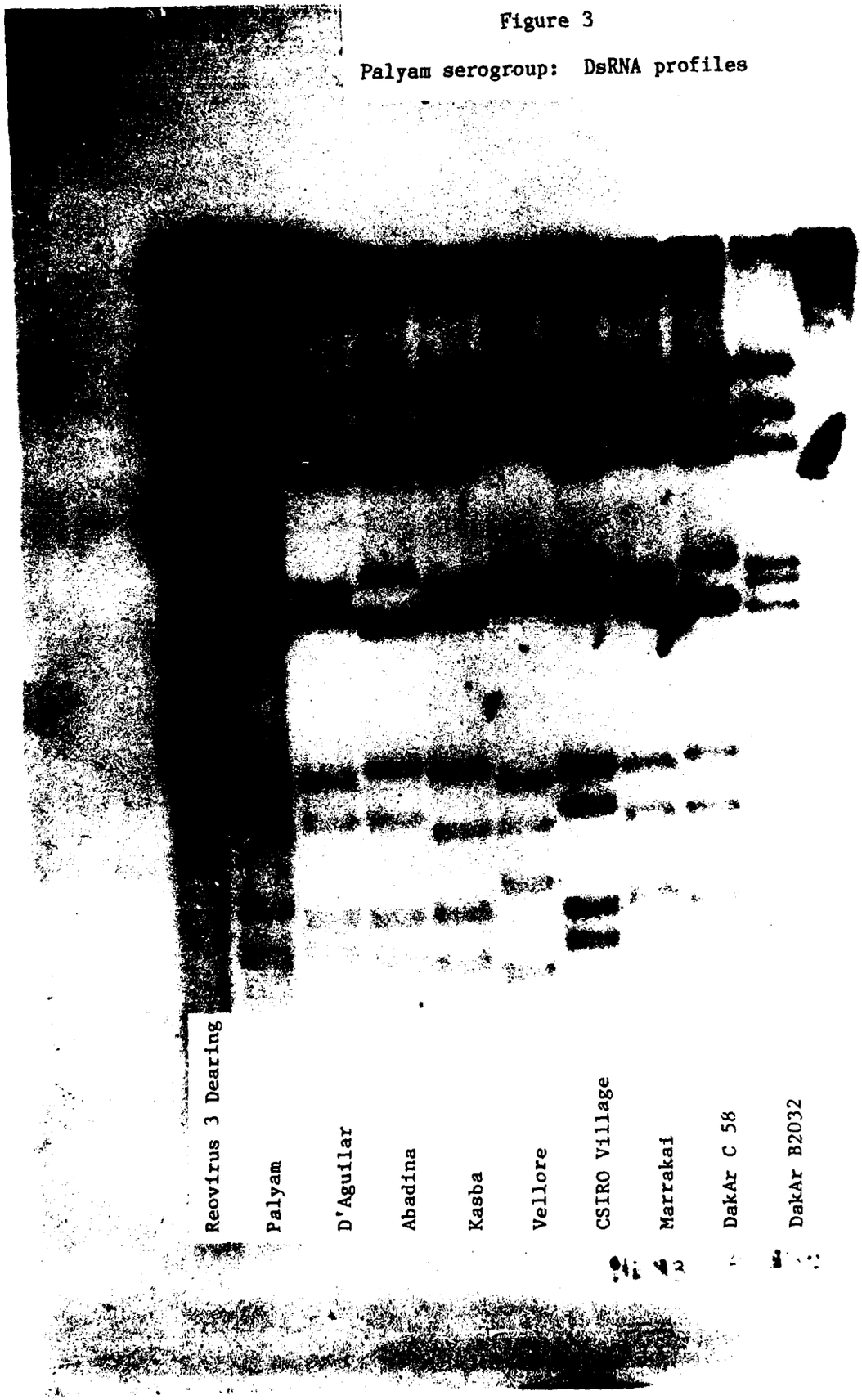
Results of plaque reduction neutralization tests with Palyam serogroup viruses

	ANTISERUM										
VIRUS	PALYAM	CSIRO VILLAGE	MARRA-KAI	BUNYIP CREEK	VELLORE	B2032	KASBA	ABADINA	K 58	D'AGUILAR	NYABIRA
PALYAM	320*	320	0	0	0	0	0	0	0	0	0
CSIRO VILLAGE	40	5,120	0	0	0	0	0	0	0	0	0
MARRAKAI	0	0	20,480	0	0	0	10	20	0	0	0
BUNYIP CREEK	0	0	0	80	640	0	0	0	0	0	0
VELLORE	0	0	0	20	10,240	0	0	0	0	0	0
DAK AR B 2032	0	0	0	0	10	10,240	0	0	0	0	0
KASBA	0	0	40	0	0	0	≥20,480	2,560	320	0	0
ABADINA	0	0	0	0	0	0	1,280	5,120	1,280	0	0
DAK AR K 58	0	0	0	0	0	0	2,560	2,560	1,280	0	0
D'AGUILAR	0	0	0	0	20	0	0	0	0	320	1,280

* Reciprocal of highest antiserum dilution producing ≥90% plaque inhibition.

Figure 3

Palyam serogroup: DsRNA profiles



Characterization of the Tete serogroup and a new member, I612045 (G. Modi and A. J. Main). This strain was isolated from the blood of a myna bird in India during 1961. It was shown to be a member of the Tete serogroup by complement-fixation (Table 14), hemagglutination-inhibition (Table 15), and neutralization (Table 16) tests. Plaque-reduction neutralization tests are incomplete.

Table 14

Complement-fixation tests comparing members of the Tete serogroup

	ASCITIC FLUIDS*				
	I612045	Tete SAAn4511	Batama DakAnB1292	Bahig EgB90	Matruh UARAn1047	Tsuruse Mag271580
I612045	512/>256	128/>256	128/≥256	64/≥256	32/8	0
Tete	128/≥128	1024/>128	512/≥128	64/≥128	32/32	16/8
Batama	128/≥128	1024/≥128	>1024/>128	64/≥128	128/64	8/≥128
Bahig	256/16	0	128/128	256/>128	256/>128	16/8
Matruh	0	0	16/16	256/≥32	256/>32	0
Tsuruse	32/>128	0	128/≥128	64/≥128	16/64	64/>128

Reciprocal of serum titer/reciprocal of antigen titer.
*initial dilution 1:8
**initial dilution 1:64 (anticomplementary at 1:32).

Table 15

Hemagglutination-inhibition tests comparing members of the Tete serogroup

	ASCITIC FLUIDS					
ANTIGEN	I612045	Bahig	Tsuruse	Tete	Batama	Matruh
I612045 (8 HA units)	≥10240*	20	10	80	160	80
Bahig (8 HA units)	160	40	40	10	160	20
Tsuruse (1 HA unit)	≥10240	40	320	20	160	20
Tete. (2 HA units)	10	<10	<10	40	320	<10

*Reciprocal of serum titer.

Table 16

Plaque-reduction neutralization tests in Vero cells comparing members of the Tete serogroup

	ASCITIC FLUID			
Virus	I 612045	Bahig	Batama	Tsuruse
I 612045	1280*	20	40	<10
Bahig	≥320	80	20	<10
Batama	10	<10	640	<10
Tsuruse	160	10	<10	160
Matruh	≥320	20	20	<10
Tete	<10	<10	<10	<10

*Reciprocal of highest dilution reducing the number of plaques by 90%.

II. IDENTIFICATION OF VIRUSES

Identification of a new phlebovirus from Italy (R.B.Tesh and A.J. Main). Virus strain ISS. Phl. 18 was received from Dr. Paola Verani, Istituto Superiore di Sanita, Rome. It is one of 4 antigenically similar viruses isolated from Phlebotomus perniciosus in central Italy in 1980-1981. Initially, ISS. Phl.18 antigen (spot slides of infected Vero cells) was screened by indirect fluorescent antibody test (IFAT) against the following mouse immune ascitic fluids: phlebotomus fever group, Naples, Karimabad, Salehabad, Toscana, Arumowot, Gordil, Saint Floris, Sicilian, Rift Valley fever, Gabek Forest, VSV group, Isfahan, Chandipura and Jug Bogdanovac. Positive reactions were obtained in the IFAT with Toscana, Naples, Gordil, Saint Floris, Salehabad, Gabek Forest and the phlebotomus fever grouping antisera.

ISS.Ph.18 antigen and antiserum were then tested by complement-fixation method against Salehabad, Gordil, Gabek Forest, Saint Floris, Toscana and Naples antigens and mouse immune ascitic fluids. Positive reactions were obtained between ISS.Phl.18, Salehabad and Gabek Forest as shown in Table 17.

ISS.Phl.18 and Salehabad viruses and antisera were then examined by plaque reduction neutralization method with the results shown in Table 18. In summary, our findings indicate that ISS.Phl.18 is a new phlebovirus which is related to but distinct from Salehabad virus.

Table 17

Results of CF test with ISS.Phl.18

	ANTISERUM		
ANTIGEN	ISS.Phl.18	Salehabad	Gabek Forest
ISS.Phl.18	256/≥256*	128/≥64	0
Salehabad	64/≥128	256/≥128	0
Gabek Forest	16/4	0	≥256/≥128

*Reciprocal of highest positive antiserum dilution/highest positive antigen dilution.

0 = <4/4

Table 18

Results of plaque-reduction neutralization tests with Salehabad and ISS.Phl.18 viruses

	ANTISERUM	
Virus	ISS.Phl.18	Salehabad
ISS.Phl.18	160*	<10
Salehabad	<10	320

*Reciprocal of highest antiserum dilution producing 90% plaque inhibition.

RML-85 (M. Fletcher and A. J. Main). Forty-one strains of virus, rickettsiae, and spiroplasms, in various stages of characterization, were received from Dr. Conrad Yunker at the Rocky Mountain Laboratory in Hamilton, Montana. Most of these isolates had been recovered from ticks. Mouse brain stocks of most viruses have been prepared and deposited in the WHO bank. One strain, RML-85 has been partially identified at YARU. This strain was isolated from the blood of 2 murre (Uria sp.) from Alaska. It was shown to be in the Great Island complex of the Kemerovo serogroup by complement-fixation (Table 19). Plaque reduction neutralization tests to determine specificity are underway.

Table 19

Complement-fixation tests comparing RML-85 with other members of the Kemerovo serogroup

	RML-85	
	Antigen	Ascitic Fluid
Kemerovo	64/256*	64/128
Tribec	64/64	64/128
Lipovnik	16/32	16/128
Cape Wrath	128/128	64/128
Okhotskiy	512/128	32/128
Tindholmur	64/32	32/128
Mykines	32/64	16/32
Yaquina Head	256/64	64/128
Great Island	128/128	64/128
Bauline	128/128	64/128
Kenai	128/32	64/128
Poovoot	256/128	32/32
Nugget	256/256	64/128
Chenuda	<16/256	<8/128
Mono Lake	<8/128	8/128
Huacho	16/128	16/128
Baku	64/>512	8/128
Sixgun City	16/128	<8/32
Wad Medani	<8/32	<8/128
Seletar	16/128	<8/32

*Heterologous titer/Homologous titer.

Conn Ar-560-79 (L. Lorenz and A. J. Main). This strain was isolated from a pool of 22 nonblooded adult female Aedes triseriatus collected between 10-14 September 1979 in light traps in North Madison (New Haven Co.) Connecticut. A sucrose-acetone extracted mouse-brain antigen failed to agglutinate goose erythrocytes but did fix complement in the presence of the homologous antibody and with Bunyamwera group ascitic fluids (Table 20). Plaque-reduction neutralization tests comparing this strain with the members of this serogroup, as well as Bunyamwera group isolates from Ohio (17 strains from Ms. Margaret Parsons of the Vector-borne Disease Unit of the State Department of Health), New Jersey (14 strains isolated at YARU from mosquitoes submitted by Dr. Wayne Crans of Rutgers University), Ontario (1 strain from Dr. Harvey Artsob of the National Arbovirus Reference Service), and Manitoba (4 strains from Dr. Artsob) are incomplete (Table 20).

Table 20

Serologic tests comparing Conn Ar-560-79 with members of the Bunyamwera serogroup

	Conn Ar-560-79 Antigen		Ascitic Fluid	
	CF	NT	CF	NT
Cache Valley (6V633)	16/16	<10/-	64/64	-
Tensaw (A9-1716)	16/32	<10/-	16/64	-
Northway (0234)	≥512/≥512	40/-	32/64	-
Wyeomyia (original)	<8/64	-	<8/64	-
Main Drain (BFS5015)	64/128	-	64/64	-
Lokern (FMS 4332)	16/16	-	16/64	-
Guaroa (352111)	<8/128	-	<8/64	-

Zim Bat Pool A/81 (A.J.Main). This strain was isolated from pooled organs from ten bats of at least two species. The virus was submitted by Dr. N.K. Blackburn of the Veterinary Research Laboratory in Causeway, Zimbabwe. It is a strain of Sindbis virus by complement-fixation, hemagglutination-inhibition, and plaque-reduction neutralization tests (Table 21).

Table 21

Serologic tests comparing Zim Bat Pool A/81 with members of the Sindbis complex of alphaviruses

	Zim Bat Pool A/81							
	Antigen				Ascitic Fluid			
	CF	HI* (1 hr)	HI* (24 hrs)	NT**	CF	HI* (1 hr)	HI* (24 hrs)	NT**
Sindbis	32/64	40/40	80/80	32/32	128/256	160/≥5120	640/≥5120	16/128
Whataroa	-	-	-	-	16/256	-	-	-
Aura	<8/32	<10/10	<10/40	-	16/256	20/≥5120	20/≥5120	-
Y 62-33	<8/16	10/40	<10/80	-	<8/256	160/≥5120	320/≥5120	-
Fort Morgan	<8/16	<10/-	<10/-	-	32/256	-	-	-
Highlands J	8/64	40/2560	40/≥5120	-	32/256	160/≥5120	640/≥5120	-
WEE	<8/32	20/80	20/640	-	8/256	40/≥5120	80/≥5120	-

*1 and 24 hour incubation periods, respectively.
**plaque-reduction neutralization tests in Vero cells with 90% end-points.

EthAr 3554 (D.L. Knudson and A.J. Main): This strain was isolated from Rhipicephalus ticks in Ethiopia by Dr. Owen Wood of the U.S.Naval Medical Research Unit #3. It was shown by PAGE analysis to have an RNA profile similar to viruses in the Kemerovo serogroup. It was shown to be a member of the Wad Medani/Seletar complex by complement-fixation (Table 22).

Table 22

Complement-fixation tests comparing EthAr 3554 with Kemerovo group viruses

	Kemerovo (R10)	Lipovnik (Lip91)	Wad Medani (Jamaican Tick)	Seletar (RudSM-214)
EthAr 3554	<8/<4	<8/<4	≥32/≥32	128/64
Kemerovo	>32/>32	-	-	-
Lipovnik	-	16/>32	-	-
Wad Medani	-	-	32/16	64/32
Seletar	-	-	16/8	128/64

*Reciprocal of serum titer/Reciprocal of antigen titer.

FrBrest/Ar/T577 and Fr Brest/Ar/T578 (A.J. Main). These two strains of virus were recovered from Ixodes ventalloi and Ixodes ricinus ticks, respectively, collected from wild rabbits (Oryctolagus cuniculi) near Laval, Mayenne, France. They were submitted by Dr. Claude Chastel of the Faculte de Medecine de Brest in France. Both isolates appear to be new members of the Kemerovo serogroup by complement-fixation tests (Table 23).

FrBrest/Ar/T261 and FrBrest/Ar/T439 (A.J. Main). These strains were isolated from Ixodes uriae collected at Cape Sizun, France, during 1979 and 1980, respectively. They appear to be in the Sakhalin serogroup, closest to the Avalon virus by complement-fixation tests (Table 24).

Tribec (D. L. Knudson, R.E.Shope, and A.J. Main). The prototype strain of Tribec virus was shown by PAGE analysis to be composed of four distinct genotypes. Ascitic fluids prepared against plaque-picked clones of each of four genotypes were tested by complement-fixation and found to be indistinguishable (Table 25).

Table 23. Complement-fixation tests comparing Brest/Ar/T577 and Brest/Ar/T578 with other members of the Kemerovo serogroup

Virus	Strain	Antigen		Ascitic Fluids	
		T577	T578	T577 (Brest)	T578 (YARU)
	Brest/Ar/T577	-	640/160	-	≥1024/512-
	Brest/Ar/T578	≥1024/512	-	640/160	-
Kemerovo	R10	<8/256	<8/256	<10/40	-
Lipovnik	Lip 91	<8/32	<8/32	<10/40	-
Tribec	original	8/128	<8/128	<10/40	-
Tribec	original (471)	<8/512	<8/512	<10/40	-
Tribec	original (472)	<8/256	<8/256	<10/40	-
Tribec	original (473)	<8/512	<8/512	<10/40	-
Tribec	original (475)	<8/256	<8/256	<10/40	-
Cape Wrath	ScotAr 20	-	-	<20/160	<8/512
	FinV-808	-	-	<20/160	<8/512
	FinV-873	-	-	<20/160	<8/512
	FinV-962	-	-	<20/160	<8/512
Poovoot	RML 57493-71	-	-	<20/160	8/512
Kenai	RML 71-1629	-	-	<20/160	8/512
Great Island	CanAr 41	-	-	<20/160	16/512
Bauline	CanAr 14	-	-	<20/160	8/512
Yaquina Head	RML-15	-	-	20/160	<8/512
Okhotskiy	LEIV 287ka	-	-	<20/160	<8/512
Tindholmur	DenAr 2	-	-	<20/160	8/512
Mykines	DenAr 12	-	-	<20/160	8/512
Nugget	AusMI-14847	-	-	<20/160	<8/512
Seletar	RudSM 214	-	-	<20/160	16/512
Wad Medani	EgAr 492	-	-	<20/160	16/512
	UK FT 363	-	-	<20/160	<8/512
Huacho	CalAr 883	-	-	<20/160	<8/512
Mono Lake	CalAr 861	-	-	<20/160	<8/512
	Brest/Ar/T222	-	-	<20/160	8/512
Sixgun City	RML 52451	-	-	<20/160	<8/512
Baku	LEIV 46A	-	-	<20/160	16/512
Chenuda	EgAr 1152	-	-	<20/160	35/512

Table 24

Complement-fixation tests comparing Brest/Ar/T439 and Brest/Ar/T261 with members of the Sakhalin serogroup

	T439	T261	AVA	CM	TAG	TILL	SAK
T439	128/>128	-	1024/16	0	0	0	0
T261	-	128/>64	512/16	-	-	4/≥64	-
Avalon	128/≥128	64/≥64	>1024/>128	-	-	-	-
Clo Mor	0	-	-	32/32	-	-	-
Taggert	0	4/4	-	-	>1024/>128	-	-
Tillamook	0	4/4	-	-	-	512/>128	-
Sakhalin	0	4/4	-	-	-	-	64/16

0 = <4/<4

Table 25

Complement-fixation tests comparing the four genotypes of Tribec virus

	ASCITIC FLUIDS				
ANTIGENS	Tribec 471	Tribec 472	Tribec 473	Tribec 475	Tribec original
Tribec-471	512/>128	128/≥128	512/128	256/128	64/≥128
Tribec-472	512/≥128	256/>128	512/≥128	256/≥128	128/≥128
Tribec-473	256/≥128	128/≥128	512/>128	256/>128	64/≥128
Tribec-475	512/≥128	256/≥128	512/≥128	256/>128	128/≥128
Tribec-original	512/≥128	256/≥128	512/≥128	256/≥128	128/>128
Normal	0	0	0	0	0

Reciprocal of serum titer/reciprocal of antigen titer.

Viruses isolated in C6/36 Ae. albopictus cells in Indonesia (J. Converse, R.E. Shope, R.B. Tesh). Fifteen agents isolated in Bali and Flores, Indonesia by Dr. Converse of the NAMRU-2 were referred for identification. Each of the agents produced CPE in either C6/36 or A. pseudoscutellaris mosquito cells in the Indonesia Laboratory. On passage at Yale, 8 of the strains produced CPE in C6/36 cells in two to four days. Three of these, JKT6468, JKT5441, and JKT8442 reacted by IFA with group B ascitic fluid. These 3 viruses plus JKT8132 killed infant mice ic. The other C6/36 fluids did not kill mice.

The C6/36 cells are an efficient means of isolating arboviruses from field material. When the agent adapts readily to mice, the identification can proceed in a routine manner. Those agents which do not adapt to mice pose a special problem in identification. Studies are underway to see if infection-immune mouse serum of these viruses can be used in IFA to establish homologous system reactions. If not, it may be necessary to identify the agents by neutralization test in C6/36 cells.

III. SEROLOGIC SURVEYS

Serologic survey of human sera from Greece (R.B. Tesh and A. Antoniadis). In collaboration with Dr. A. Antoniadis, Aristotelian University of Thessaloniki, a total of 245 Greek human sera was examined by plaque reduction neutralization test (PRNT) for antibodies to Naples and Sicilian sandfly fever viruses. These same sera had previously been examined by HI test for antibodies to Rift Valley fever virus. Twenty-four of the sera had reacted in HI test with positive titers of 1:10 to 1:20 with Rift Valley fever antigen. Results of PRNT with Naples and Sicilian viruses are shown below.

Locality (county)	Total Sera tested	Naples* positive	Sicilian* positive
Erros	58	8	2
Imalhia	36	8	1
Magnisia	49	1	1
Lakonia	23	4	0
Chania	27	5	1
Iraklio	46	15	0

*Sera screened at a 1:10 dilution. Specimens producing 90% plaque inhibition were recorded as positive.

Of the 24 Rift Valley fever positive sera, 14 reacted in PRNT with either Naples or Sicilian virus. The remaining 7 sera did not neutralize either agent, however. Since Rift Valley fever is not known to occur in Greece, the most likely explanation for the remaining 7 positive sera is that they represent cross-reactions with antibodies to a phlebovirus other than Naples or Sicilian. Further studies are necessary to identify this agent(s).

Survey of Egyptian and Sudanese sera for antibody to arboviruses (J.M.Meegan, G. Tignor). The two separate areas of study in this project are a) determination of the prevalence of antibodies to the hemorrhagic fever viruses -- Lassa, Marburg, RVF, Crimean-Congo hemorrhagic fever (CCHF) and Ebola (Zaire and Sudan strains) (EBO-Z and EBO-S), and b) determination of prevalence of antibodies to 15 other arthropod-borne viruses which have been implicated in human disease. The study of hemorrhagic fevers was supported by a US Army contract other than the Reference Center and will be reported separately. The second part of this study was evaluating antibody prevalence for arthropod-borne viruses. Over 1000 sera have been screened by IFA tests against 15 viral agents, and those reacting positively are being retested in microneutralization tests. The neutralization studies have confirmed our initial IFA studies indicating countrywide circulation of flaviviruses (especially West Nile) at antibody prevalence rates of 20%. Less prevalent (2-8%) are antibodies to alphavirus, Bunyamwera virus, phleboviruses, and a variety of ungrouped arthropod-borne viruses implicated in human disease.

IV. DIAGNOSIS OF DISEASE

Diagnosis of CNS cases in Connecticut (A.J. Main). Acute and/or convalescent sera from four Connecticut residents were submitted for arboviral serology by Dr. Douglas Moore and Ms. MaryAnn Markowski of the State Department of Health Services. All samples were negative by HI and/or CF with the following antigens: EEE, WEE, Highlands J, SLE, Powassan, Connecticut, snowshoe hare, Jamestown Canyon, California encephalitis, Keystone, La Crosse, and Cache Valley.

A single acute serum was received from Dr. Mary Jean Ahern of the Griffin Hospital in Derby, Connecticut. The patient had recently returned from Puerto Rico with clinical dengue. Attempts to isolate virus or demonstrate HI antibody to each of the four dengue serotypes were unsuccessful.

Serologic diagnosis of febrile illnesses among gravely ill pediatric patients in a Jakarta hospital (G.J. Olson, J. Boshell). During 1978 and 1979 pediatric patients were selected for study to determine if Japanese encephalitis (JE) virus was responsible for meningitis and encephalitis occurring in Sumber Waras Hospital, Jakarta, Indonesia. Further, the study was designed to estimate the relative importance of arthropod-borne viruses in this series of seriously ill pediatric patients.

A total of 108 subjects was studied who had clinical diagnoses of encephalitis and from whom acute and convalescent phase serum specimens were collected. Each pair of sera was tested by hemagglutination inhibition (HI) test for evidence of flaviviral infection. Of the 10 petients who showed fourfold increases in antibody from the acute to the convalescent phase, 7 showed diagnostic rises in neutralizing antibodies to dengue (DEN) virus (Table 26). Three of the 10 showed no evidence sero-conversion to any of the flaviviruses used in the test (DEN, JE, and Zika).

Our studies continue to determine the relative importance of other viral agents as cause of illness in this patient population. Measles, mumps, lymphocytic choriomeningitis, varicella, herpes and cytomegalo-viruses and other arthropod-borne viruses will be studied. However, preliminary studies completed suggest that at least 6% of the pediatric encephalitis in this Jakarta hospital population was caused by DEN and that JE was not responsible for the pediatric encephalitis which occurred.

Table 26

Serologic test results of encephalitis patients with evidence of flaviviral infection

Serum		HI (JE) titer	NT titer DEN-1	DEN-2	DEN-3	DEN-4	JE	Zika
10314	A	>640	-	160	80	20	<5	<5
	C	>640	-	>640	80	160	<5	<5
10469	A	<10	<10	<10	<10	<5	<5	<5
	C	160	<10	40	<10	10	<5	<5
10516	A	20	<5	20	<5	<5	<5	<5
	C	160	5	>320	10	20	<5	<5
10587	A	40	<20	<10	<10	<5	<5	<5
	C	640	<5	100	<100	<5	<5	<5
10590	A	320	<5	<5	<5	<5	<5	<5
	C	320	<10	<5	<5	<5	<5	<5
10595	A	<10	<20	<20	<20	5	<5	<5
	C	80	20	20	40	5	<5	<5
10629	A	160	<20	40	40	40	<5	<5
	C	160	<5	160	40	>640	<5	<5
10751	A	<10	-	-	-	<5	<5	<5
	C	40	20	20	40	<5	<5	<5
10787	A	<10	-	<40	<4	<5	<5	<5
	C	80	-	80	80	20	<5	<5
10788	A	<10	-	<40	<40	<4	<5	<5
	C	20	-	<40	<40	<4	<5	<5

Retrospective Studies on U.S. Naval Station Populations, Guantanamo Bay, Cuba after the Cuban dengue epidemic of 1981 (Capt. C. J. Maas, MC, USN, Ens. J.G.Gee, MSC, USNR, and J.G.Olson). The last epidemic of dengue fever in Cuba began in 1977 and lasted into 1978. A total of 553,132 clinical cases were reported and an estimated 5 million people infected. Following the 1977-78 epidemic which was caused by the dengue 1 serotype, the incidence of dengue fever was sporadic with 1,497 cases reported in 1979 and 169 in 1980.

In June 1981, the reported number of dengue fever cases exceeded 1,000 per day. Reported numbers of cases increased until July 6, when the numbers of cases per day was 11,721. The last case occurred on October 10 and the epidemic was declared over on November 19th. During the epidemic 344,203 cases were reported with 158 deaths. More than 100 deaths were due to the severe forms of dengue infection known as dengue hemorrhagic fever and dengue shock syndrome and had occurred in children under 15 years of age. Incidence rates of reported cases were similar for all age groups. Infants under one year of age experienced 24.1 cases per 1,000; 1-4 year-olds had 24.5 per 1,000; 5-14 year-olds had 25.8 per 1,000 and those 15 years of age and older experienced 28.6 cases per 1,000 population at risk. The virus responsible for this epidemic has been identified as dengue-2 serotype.

Objectives of our study

We wanted to determine whether the dengue fever outbreak in Cuba had resulted in clinical or subclinical dengue infections among the personnel of the U.S. Naval Station at Guantanamo Bay, Cuba. We contacted Captain C.J. Maas, Commanding Officer of the U. S. Naval Hospital, Guantanamo Bay who had no reports of dengue-like illness during the epidemic period. We arranged to conduct a seroepidemiologic study of the personnel and obtained data on vector control operations and surveillance. Four subpopulations were sampled: Cuban nationals who live off the base but commuted to work on a daily basis, U.S. personnel who experienced illnesses during this epidemic period, U.S. personnel who because of their occupations were at great risk of being bitten by mosquitoes and U.S. personnel whose occupations made mosquito contact less likely.

Methods

Hemagglutination inhibition tests were completed on all sera received using dengue-2 hemagglutinating antigen using the method of Clarke and Casals. Subjects whose sera had 1:10 titers or greater of dengue-2 hemagglutination inhibiting antibody were considered to have had a flavivirus infection at some time. All sera positive by HI test were then tested by microneutralization for antibodies to dengue-2 virus. The presence of dengue-2 neutralizing antibodies in serum specimens suggests that these subjects may have been infected during the 1981 epidemic. Subjects were informed about the purpose of the study and gave their consent to be included. Venous blood specimens were obtained, centrifuged and frozen serum transported to the Yale Arbovirus Research Unit, New Haven, CT where all laboratory tests were performed.

Morbidity data routinely reported by the U. S. Naval hospital was obtained in order to evaluate whether the incidence of illnesses which might be confused with dengue fever had increased during the epidemic period.

Results

Figure 4 represents the dengue fever cases reported by week in Cuba during 1981 (Epidemiological Bulletin, PAHO 3(1), 1982). The outbreak began in June, peaked early in July and was over by October. Figures 5 and 6 show that there was no obvious excess morbidity at the U.S. Naval Station, Guantanamo Bay during the months of the epidemic. Table 27 shows the results of serologic testing with DEN-2. Persons who experienced febrile illnesses had a statistically significantly greater (24%) prevalence of DEN-2 neutralizing antibody than either control population. These data are difficult to interpret since there was no evidence of when the 6 clinically ill patients whose sera indicated a DEN infection were infected. The interpretation of the 95% prevalence in the Cuban community population is confounded by the fact that persons infected with more than one serotype of DEN may exhibit cross-reactive antibody. Further studies are underway to determine whether the infections are recent and to determine the DEN serotype responsible using IgM assays. Travel histories of the patients who had evidence of DEN infections have been requested in order to determine if infection could have been acquired elsewhere.

Table 27

Serologic evidence of dengue infection in Guantanamo Bay, Cuba, 1982

Population	No. positive* / No. Tested (percent positive)	
	hemagglutination inhibition test	neutralization test
Cuban commuters	74/77 (96%)	73/77 (95%)
U.S. personnel with history of illness during epidemic	8/25 (32%)	6/25 (24%)
U.S. personnel occupationally exposed to mosquito bites	1/25 (4%)	0/25
U. S. personnel without occupational exposure	3/62 (5%)	2/62 (3)

*Antibody titers 1:10 were considered evidence of previous infection.

Figure 4

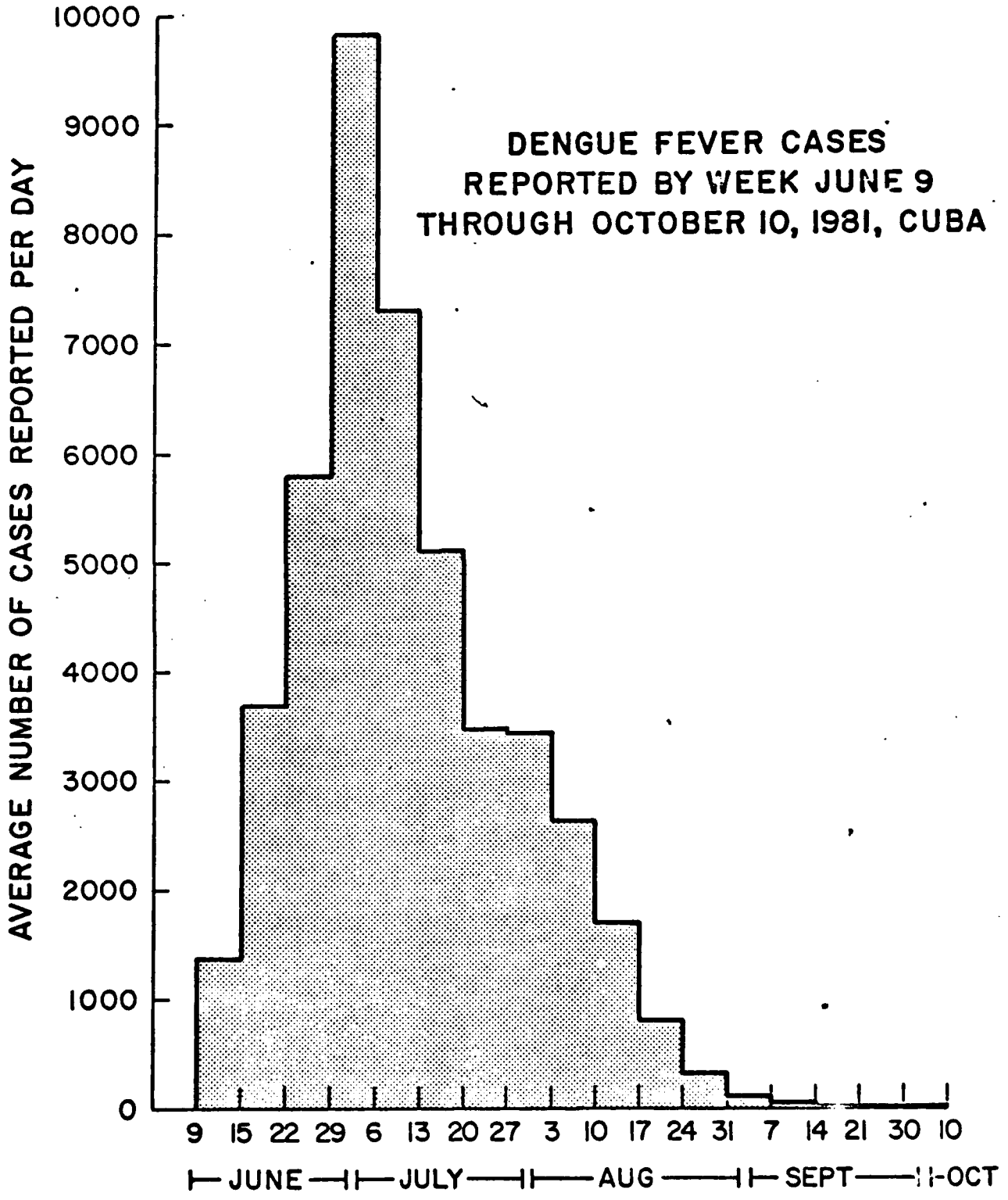

DENGUE FEVER CASES
REPORTED BY WEEK JUNE 9
THROUGH OCTOBER 10, 1981, CUBA
AVERAGE NUMBER OF CASES REPORTED PER DAY
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
9 15 22 29 6 13 20 27 3 10 17 24 31 7 14 21 30 10
JUNE
JULY
AUG
SEPT
OCT

Figure 5

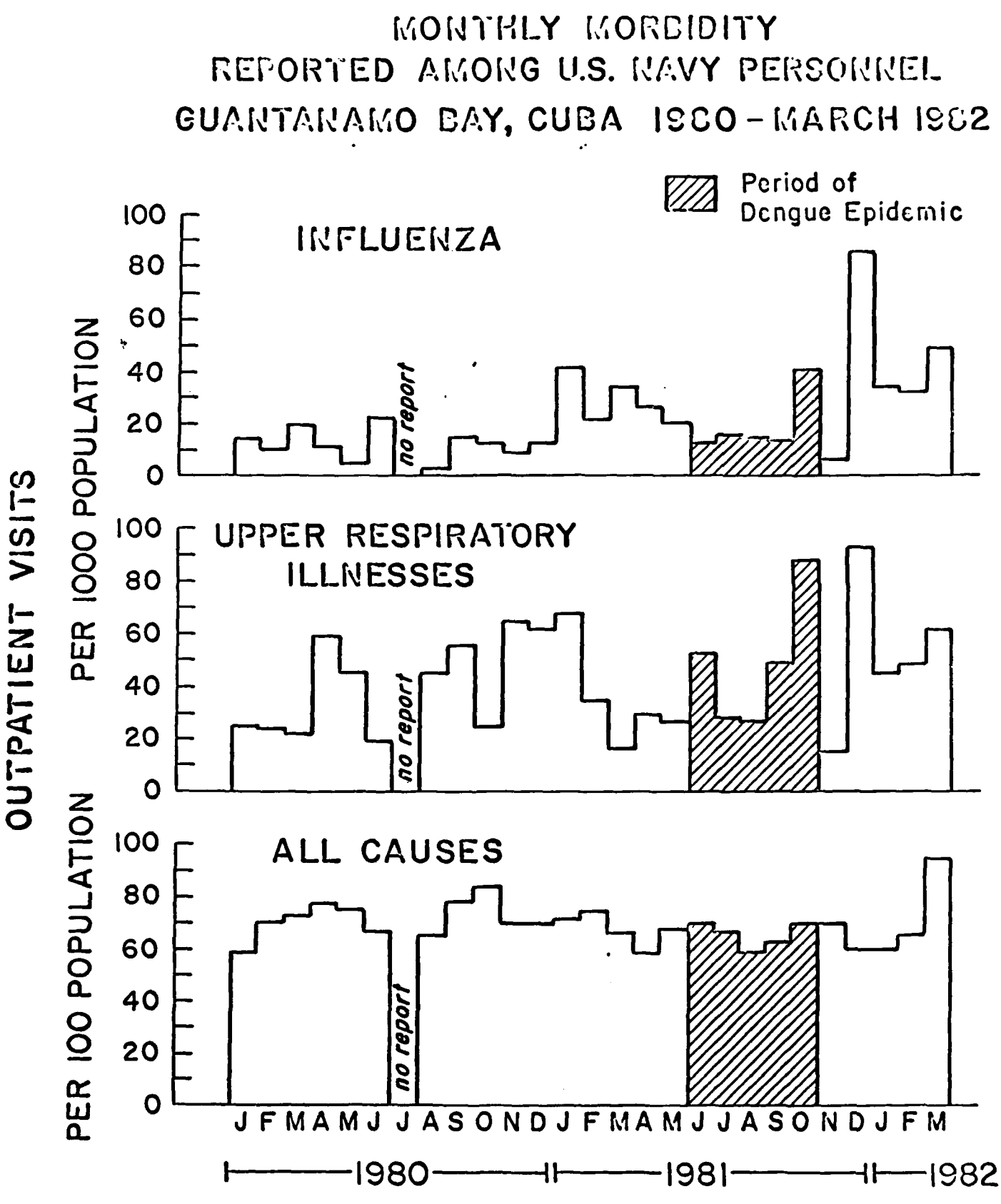
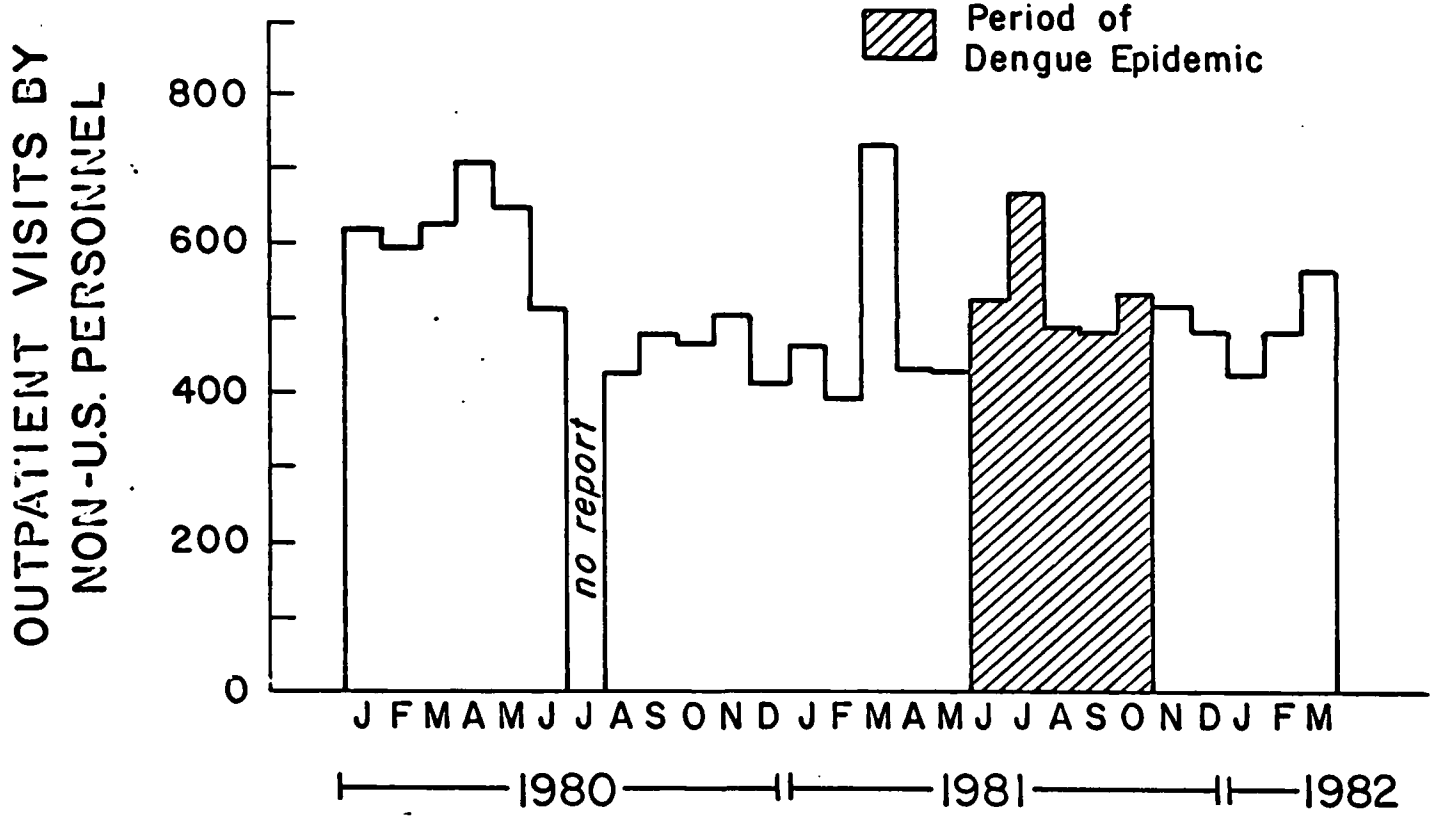

MONTHLY MORBIDITY
REPORTED AMONG U.S. NAVY PERSONNEL
GUANTANAMO BAY, CUBA 1980 - MARCH 1982
Period of Dengue Epidemic
OUTPATIENT VISITS
PER 1000 POPULATION
INFLUENZA
UPPER RESPIRATORY ILLNESSES
ALL CAUSES
PER 100 POPULATION
no report
0
20
40
60
80
100
J F M A M J J A S O N D J F M A M J J A S O N D J F M
1980
1981
1982

Figure 6

MONTHLY MORBIDITY REPORTED IN NON-U.S. PERSONNEL* GUANTANAMO BAY, CUBA 1980 – MARCH 1982



* population at risk was not reported.

Rift Valley fever antibody persisting for 49 years after infection (C.J. Peters, J.M. Meegan, R.E. Shope). A laboratory technician, G.W.M., working for Dr. Max Theiler at the Rockefeller Foundation Virus Laboratories, New York developed a laboratory infection with Rift Valley fever virus. His illness which started on Monday, October 9, 1933 is documented in Kitchen, SF, Am. J. Trop. Med. 14:547-564, 1934. Rift Valley fever was isolated from his acute phase serum and protective antibodies were demonstrated 2 weeks later. G.W.M. subsequently worked with the Lunyo strain of Rift Valley fever in the 1950's. He moved to Yale in 1964 and continued to work, but not with RVF virus, until retirement in 1975. He was re-bled June 16, 1982 in order to find out how long Rift Valley fever antibody persists.

Table 28 shows results of neutralization, IFA, and ELISA tests of serial bleedings between 1952 and 1982. Surprisingly he has maintained high titers -- up to 1280 by PRNT -- in each method used for testing. The ELISA results indicate that the antibody is not primarily IgM and is presumably therefore IgG.

RVF neutralizing antibody has been previously recorded to persist for at least 12 years (Sabin and Blumberg. Proc. Soc. Exp. Biol. Med. 64:385-389, 1947) but this is the first demonstration of persistence for 49 years.

Table 28

Rift Valley fever antibody of patient GM over a 49 year period

	Plaque reduction NT*			ELISA	
Date	Lunyo	ZH501	IFA	Ig	IgM
01/16/52	640	640	320	-**	-
11/10/53	320	640	320	-	-
10/25/57	160	640	80	-	-
06/04/58	320	640	160	-	-
02/01/63	320	320	160	-	-
01/29/65	640	640	320	800	<200
06/09/69	640	640	320	400	<200
04/23/75	1280	1280	320	800	<200
06/16/82	640	640	160	-	-

*80% plaque reduction; PRNT and IFA done at USAMRIID.
**Not tested

Diagnosis of flavivirus encephalitis in a Canadian patient exposed in Manchuria (H. Artsob, D. Burke, R.E. Shope, J.M. Meegan, R.B. Tesh). Three sera of patient H.B. were referred to Yale from the Canadian National Arbovirus Reference Service. H.B. developed encephalitis immediately on her return to Canada from Manchuria with a brief stopover in Hong Kong. A brain biopsy showed no evidence of herpes. Test results to date are shown in Table 29.

Table 29

Serological reactions of suspected JE encephalitis patient

Sera	JE	SLE		DEN		POW		MAC ELISA		
	PRNT	HI	CF	HI	CF	HI	CF	JE	POW	SOK
Sept 2 '82	160	80	4	10	0	0	0	+	-	+
Sept 15 '82	80	80	4	10	0	0	0	+	-	+
Sept 29 '82	80	80	0	10	0	0	0	+	-	+

There is clear evidence, especially with the positive IgM antibody capture (MAC) ELISA performed at Yale by Dr. Donald Burke, that the patient had a flavivirus infection, probably JE virus. The MAC ELISA cross-reaction with Sokuluk virus needs to be explored further for definitive diagnosis of this illness.

Febrile illness in Spanish workers exposed in Libya (R. Najera, R.E. Shope). Eight sets of paired sera from Spanish laborers, evacuated to Madrid from Libya with severe febrile illness were referred for testing by the Centro Nacional de Microbiologia, Madrid. The sera were tested and found negative by HI with dengue and Rift Valley fever antigens. The cause of the illness remains undetermined.

V. DEVELOPMENT OF NEW TECHNIQUES

The enzyme-linked solid phase immunoassay for antibody (IgG and IgM) and antigen detection (J. Meegan).

The enzyme-linked immunosorbent assay (ELISA) has been used for several serological groups (see previous Annual Reports). We have developed an ELISA for the detection of antigen or antibody to Rift Valley fever (RVF) virus. The ELISA for antibody detection was developed using inactivated antigens. Virus bands recovered after ultracentrifugation (potassium tartrate-glycerol gradients) of either formalin inactivated tissue culture vaccine or infected mouse liver yielded excellent ELISA antigens. After binding the antigen to 96-well plates, the test proceeded with the sequential addition of: human serum, goat anti-human serum conjugated with alkaline phosphatase, and finally enzyme substrate. The test for antigen detection involved binding mouse anti-RVF virus antisera to 96-well plates followed by the sequential addition of: presumptive viremic serum, rabbit anti-RVF virus antisera, goat anti-rabbit antisera conjugated with alkaline phosphatase, and enzyme substrate. We also employed for antigen detection a method which did not involve antibody capture of antigen. This competitive method utilized a reference anti-RVF virus serum which gave a specific titer in our standard antibody detection ELISA. We then added the suspected RVF viremic sera to the reference antisera, and retitered the sera to determine if there were RVF virus antigen in the suspect sera which bound reference antisera and lowered its titer in the antibody detection ELISA.

Studies continued on the use of the ELISA for the detection of antibodies to RVF virus. The test had been developed and perfected during past years, and initial studies revealed good correlation between the ELISA and plaque-reduction neutralization (PRNT) tests. There is a statistically significant correlation between ELISA titers and titers found in the hemagglutination-inhibition (HI) and plaque reduction neutralization (PRNT) tests (See 1981 Annual Report). These studies employed Egyptian sera collected 6 months after the epidemic of 1977. ELISA antibodies appear at day 4 post onset of RVF disease (See 1981 Annual Report), and are thus detected earlier than antibodies measured by all other methods.

To study the usefulness of the ELISA to detect antibody after vaccination, 54 samples representing pre- and post-RVF immunization sera were evaluated in a blind study to correlate PRNT and ELISA antibody titers. Previous studies comparing the complement-fixation (CF) test, IFA test and the HI test to the PRNT showed poor correlation with post-immunization sera. The ELISA correlated with the PRNT with all except for three sera. All three sera were post-immunization; one was negative by ELISA and titered 10 by the PRNT, and two were positive in ELISA but titered less than 10 by the PRNT. These results indicate that the ELISA could be used for rapid screening of large numbers of sera to determine immunization status to RVF virus.

Studies using hyperimmune mouse sera revealed the ELISA detects cross-reacting antibodies in animals immunized with a number of phleboviruses (Table 30). In each instance where a cross-reaction was detected in the ELISA, that same antisera cross-reacted in one or more standard serological test. Thus, this ELISA is one of the broadest

Table 30

Cross-reactions encountered with antisera to phleboviruses tested in the Rift Valley fever antibody detection ELISA

Antisera	Titer	
	Homologous* CF	RVF virus ELISA
Aquacate	256/128	0
Alenquer	256/32	100
Anhanga	256/8	100
Arumowot	256/128	200
Belterra	256/128	1600
Buenaventura	256/128	0
Bujaru	256/128	200
Cacao	256/128	100
Caimite	256/8	0
Candiru	256/128	400
Chagres	256/128	800
Chilibre	256/128	0
Frijoles	256/32	400
Gabek Forest	256/128	0
Gordil	256/32	1600
Icoaraci	128/128	1600
Itaituba	256/128	100
Itaperanga	256/8	400
Joa	256/128	1600
Karimabad	32/128	200
Munguba	256/128	100
Naples	256/32	200
Nique	256/8	200
Oriximina	256/128	0
Pacui	256/32	0
Punta Toro	256/128	400
Rio Grande	256/8	200
Saint Floris	256/32	800
Salehabad	256/128	0
Sicilian	256/32	0
Tehran	256/128	0
Toscana	256/128	0
Turuna	256/32	0
Urucuri	64/32	0

*Supplied by Dr. R. B. Tesh

cross-reacting tests available. Human convalescent sera from patients infected with sandfly fever-Naples (but not those infected with Sicilian) showed this same cross-reactivity in the RVF ELISA. In a series of collaborative studies, we have compared the ELISA to the PRNT using field collected sera from Egypt, Sudan, and Greece to determine if this broad cross-reactivity will be a major problem. To date, only in the collection from Greece did we find sera negative in PRNT but positive in ELISA. Most likely this represents the circulation of a cross-reacting virus. Although phleboviruses circulate in Egypt and Sudan, they are either not cross-reactive (e.g. sandfly fever-Sicilian), are cross-reactive but at lower titers and were not scored as positsive in the RVF ELISA, or are limited in distribution and not represented in the collections tested. The broad cross-reactivity of the RVF ELISA will undoubtedly be a continuing problem and attempts should be made to develop a more specific ELISA antigen perhaps from individual virion glycoproteins or protein subunits.

We have developed an ELISA method for measuring RVF virus specific immunoglobulin M (IgM) based on the isolation of IgM antibody by reaction with a 96-well plate coated with antihuman IgM. The measurement of specific IgM was accomplished by the subsequent addition of RVF virus or control antigen, monoclonal anti RVF virus antibody, and enzyme labelled antimouse antibody. The use of monoclonal antibodies substantially reduced background readings when compared to use of a polyvalent enzyme-labelled anti RVF virus. We compared the sensitivity and specificity of this method with those of a conventional form of ELISA in which RVF virus antigen is bound to the plate followed by the patient's serum, and then an enzyme-labelled antihuman IgM antibody. Titers obtained with the indirect monoclonal method correlated to a highly significant level with titers obtained with the direct method. Additionally, contrary to the direct method, the indirect method did not yield false positive results when rhematoid factor was present in the test serum.

Monoclonal antibodies to Rift Valley fever virus (J. Meegan).

Monoclonal antibodies will be utilized not only for exploring the antigenic sites on phlebotomus fever group viruses but also as a source of large quantities of purified antibodies for use with ELISA and other rapid diagnostic tests. Monoclonal antibodies are produced from hybrid cells formed by fusion of a myeloma cell line with spleen cells from an immunized mouse. Briefly, the method involves immunization of C57 BL/6 mice with 2-3 weekly doses of viral antigen mixed with equal volumes of adjuvant. Three days after the final booster, spleens are removed from immunized mice and fused (using polyethylene glycol 1500) with BALB/C NSI/1 myeloma cells at a 4:1 ratio. In media containing hypoxanthine, aminopterin, and thymidine the parental myeloma cells are not viable. Parental non-fused spleen cells will not grow in cell culture. Thus, only hybridomas grow, and supernatants from these can be tested for antibody. Hybridomas producing specific antibodies can then be maintained in cell culture or inoculated i.p. in isogenic mice with the resulting ascitic fluids containing monoclonal antibody.

In an attempt to maximize the variety of antigenic sites to which monoclonal antibodies could be produced, we employed a number of different inactivated RVF virus antigens. Crude and purified antigen preparations from cell culture-produced vaccine and infected mouse liver were utilized in their native configuration or treated with detergent. A number of hybridoma cells secreting antibody measurable by the ELISA have been produced (see 1981 Annual Report).

Our desire to obtain monoclonal antibodies to the external glycoproteins of the virion prompted us to select for further study those hybridomas produced from mice immunized with whole virion preparations from the formalin treated vaccine. Additionally, since our most abundant ELISA antigen was a purified mouse liver preparation, work with monoclonal antibodies produced with vaccine antigen would eliminate detection of non-virion antibody.

All hybridomas were cloned twice using soft agar techniques (those few hybrids which did not grow well on soft agar were cloned by limited dilution). Cells were then grown in quantity, and portions frozen in liquid N_2 and inoculated in pristane primed mice to produce ascitic fluid.

Table 31 lists the monoclonal ascitic fluids which have been tested to date by ELISA, IF, HI, and PRNT. The PRNT was performed by Dr. C.J. Peters of USAMRIID.

All of the ascitic fluids were high titered in the ELISA, but varied in their reactivity in the PRNT, HI, and CF tests. All which were neutralization positive were positive in the HI. Three were reactive at low levels in the HI but negative in the PRNT test. Only one was CF positive. Some reacted only in the ELISA. Table 32 reveals the cross reactions encountered in the IFA test using those monoclonals. Most are extremely specific. Using an ELISA test, all monoclonal antibodies were found to be of the IgG class, with eleven G_{2a}, two G_{2b}, and three G_1 isotypes (Table 33).

The proteins to which the monoclonals bind were determined by immunoprecipitation using detergent disrupted virus, then polyacrylamide gel electrophoresis. These studies are continuing, but to date, most of the monoclonals bind to the surface glycoproteins and one (the CF positive monoclonal) binds to the nucleocapsid protein (N).

Competition ELISA studies using unlabeled monoclonals to block attachment of an enzyme labeled monoclonal allowed us to topologically map the antigenic sites on the surface of the virion. We have discovered at least six antigenic sites. Attachment of antibody to four of the sites neutralizes the virus.

Table 31

Antibody titers of monoclonals to RVFV

	Titers			
Monoclonal	ELISA	NT(E)	HAI	CF
1-20-9C	$2.2X10^5$	2560	4000	<10
4-8-10C	$6.6X10^6$	2560	$4X10^4$	<10
4-10-10A	$2.2X10^6$	1280	4000	<10
4-10-3C	$2.2X10^6$	2560	4000	<10
4-39-CC	$2.2X10^6$	80	4000	<10
4-32-8D	$1.8X10^7$	$4X10^4$	4000	<10
1-25-6A	$6.6X10^5$	$8X10^4$	$4X10^4$	<10
1-25-1B	$2.0X10^6$	$4X10^4$	$4X10^4$	<10
1-21-3B	$2.2X10^6$	<10	<10	<10
1-4-3P	$2,2X10^6$	<10	<10	1000
1-29-5B	$2.2X10^6$	<10	40-80	<10
1-29-5F	$2.2X10^6$	<10	40-80	<10
1-29-4B	$6.0X10^6$	<10	40-80	<10
4-10-8B	$1.8X10^7$	NT	<10	<10
4-8-11B	$6.6X10^6$	<10	<10	<10
4-8-2B	$1.8X10^7$	<10	<10	<10

Table 32

Specificity of RVFV Monoclonals by IFA

		ANTIGENS							
Monoclonal	RFV	BTA	CHG	FRI	ICO	JOA	KAR	MUN	OTHERS**
143P	10^4	+	+	-	-	-	-	+	-*
Bl-12***	10^4	+	-	+	+	+	+	-	-
Others-25	$10-10^5$	-	-	-	-	-	-	-	-

* + = >250; - = <40

** AUR, FG, GOR, SFS, SFN, SAF, SAL, ALE, ANH, BUJ, CAC, CDU, ITA, NIQ, PT, RG, TER, TOS, UTU. Underlined viruses have demonstrated a cross-reaction with RVF virus in a standard serological test.

***Monoclonal supplied by Dr. J. Dalrymple, USAMRIID.

Table 33

ELISA to determine RVFV monoclonal antibody isotype

	O.D. 405					
Monoclonal	G1	G2A	G2B	G3	M	A
4-10-3C	.10	.56	.29	.14	.04	.04
4-10-10A	.14	.59	.20	.19	.11	.02
1-20-9C	.20	.59	.29	.17	.04	.09
4-8-10C	.14	.58	.19	.10	.05	.05
4-8-11B	.17	.85	.16	.09	.10	.05
4-10-8B	.12	.88	.18	.06	.05	.09
1-29-5F	.15	.67	.26	.11	.16	.07
4-8-2B	.20	1.19	.25	.10	.03	.02
1-29-4B	.15	.61	.25	.12	.06	.02
1-29-5B	.17	.53	.20	.09	.06	.03
4-32-8D	.15	.54	.32	.09	.02	.06
1-4-3P	.29	.20	1.31	.21	.01	.08
4-39-CC	.11	.10	.53	.06	.05	.04
1-25-1B	.36	.03	.06	.07	.03	.07
1-21-3B	1.09	.09	.09	.05	.02	.02
1-25-6A	.39	.05	.07	.07	.03	.04

Crimean-Congo hemorrhagic fever virus ELISA. (J.M. Meegan A. Antoniades, and R.E. Shope) The methodology used to develop antigens for the RVF antibody detection ELISA was employed to prepare an inactivated antigen for Crimean-Congo hemorrhagic fever (CCHF) virus. The CCHF ELISA reliably detected antibodies in hyperimmunized mice, and when tested in preliminary studies of sera from residents of Greece, it correlated well with the HI and IF tests. Further studies are planned in collaboration with Greek colleagues studying an endemic CCHF site in northern Greece.

Latex bead technique for rapid antibody or antigen detection. (J.M. Meegan) A pilot study was undertaken to evaluate the usefulness of antigen or antibody covalently bound to latex beads as a means of rapid antibody or antigen detection. Such methods are being used more frequently especially by commercial companies. We evaluated, as a prototype for arthropod-borne viruses, an 8-minute, latex-agglutination rubella antibody detection kit. In comparative tests with the HI, ELISA, and PRNT, it proved accurate, rapid, and more sensitive than the HI test. Future studies should explore the use of such solid phase spheres for antibody and antigen detection with arthropod-borne viruses.

Yellow fever virus studies with nectar meals and mosquitoes (T.H.G.Aitken, B.J.Beaty and L. Lorenz). Female mosquitoes, when not taking blood meals, and males maintain themselves in nature by feeding on flower nectar. One of the spin-off ideas resulting from the 1978 yellow fever (YF) transovarial virus transmission studies was the possibility that infected mosquitoes could contaminate flower nectaries and a subsequent nectar feeder of either sex might acquire an infection. Previous observations covering several years in this laboratory indicated that male mosquitoes are readily infected by feeding on artificial meals containing YF virus; in fact they appear to be more susceptible than female mosquitoes. Furthermore, other studies by us (Beaty and Aitken, Mosq. News 39(2):232, 1979) demonstrate that infected female mosquitoes are capable of in vitro transmission of YF virus to artificial meals of 10% fetal calf serum (FCS) and 10% sucrose. Thus the stage was set to try and demonstrate the artificial up-take of virus by male and female mosquitoes feeding on an artificial nectar meal previously exposed to an infected mosquito.

.Experiment #1:

Aedes aegypti (Amphur$_{F23}$) females were inoculated intrathoracically with 20% BMB YF virus (Asibi) titering 10.75 log 10 TCID50/ml (C6/36 cells). After an incubation period of 17 days at ca. 28°C and 75-90% RH, 2 "donor" females each (starved 39 hours) were confined to 19 tubes provided with nylon netting on which was placed a drop (0.1 ml) of nectar (10% honey, 10% FCS and 80% buffered water pH 7.5). Exposure to the meal varied from 1 3/4 to 2 hours after which the mosquitoes were removed and subsequently tested for infectivity by direct fluorescent antibody microscopy; all were strongly positive for Y.F. antigen by head squash examination. Most mosquitoes fed to repletion but at least one fed in each tube.

Following withdrawal of the "donor" females, clean "recipient" mosquitoes (2 of each sex) were introduced into each tube and allowed to feed for 15 - 20 minutes on the contaminated nectar drop. Thereafter the engorged "recipients" were confined to cages to incubate "virus" for 14 days at which time they were harvested and head and abdomen squashes prepared, stained with specific YF conjugate and examined by FA microscopy. In 13 successful tests of one or more feeding "recipient" mosquitoes, 21 males and 23 females were negative. That is they failed to produce a demonstrable YF infection.

Experiment #2:

Aedes aegypti (Amphur$_{F23}$) females were inoculated as above and allowed to incubate virus for 8 days. Thereafter 2 "donor" females each (starved 48 hours) were confined to tubes and allowed to engorge on a nectar droplet for 43 minutes to 1 1/2 hours. Most mosquitoes fed to repletion, but at least one fed in each tube. The "donors" were then removed and all subsequently shown to be infected by head squash. "Recipient" mosquitoes (2 of each sex) were then introduced into each tube to feed on the nectar droplet for about 40 minutes (note: about double the time in Exp. #1). Engorging "recipient" mosquitoes were then permitted to incubate "virus" for 21 days (rather than 14 days in the previous experiment) on the chance that small amounts of ingested virus might have a longer time to replicate and be demonstrable. In 33 successful tests, 57 male and 54 female "recipients" proved negative.

An attempt was made to recover all test nectar meals which had not dried. These were inoculated into 6-day old clean Amphur females and held at 30°C for 21 days. Meal recipients varied from 2 to 11 females depending on the size of the inoculum. Eight meals were thus tested and 3 were shown to contain YF virus derived from feeding "donor" mosquitoes. The virus recovery rate could well have been greater had the inoculated mosquitoes survived better. Mortality caused by the concentrated honey nectar was high. In any event, virus was surviving in the nectar meal for at least 4-5 hours in the 3 successful instances. There is thus still a tenuous possibility that besides transovarial transmission of virus, mosquitoes might acquire virus through nectar feeding and provide a means of virus survival through adverse climatic conditions. Nectar feeding of an infectious nature might be significant under certain rural epidemiological situations where the virus infection of mosquitoes is concentrated in a small area and there are few flowers as a source of nectar meals (supplemental in the case of haemophilic females).

The effect of mosquito colonization on susceptibility to yellow fever virus infection (T.H.G.Aitken, L. Lorenz and R.B.Tesh). Studies were undertaken to determine what effect insectary colonization of a mosquito population might have on the susceptibility to yellow fever (YF) virus infection of that population. A strain of Aedes aegypti from Vero Beach, Florida was obtained (courtesy of Dr. J. K. Nayar) and 7 filial generations of adults were simultaneously exposed to an oral meal of YF virus.

Asibi virus was grown in C6/36 cells for 6 days following which it was harvested and a meal prepared of virus (2.5 ml), washed human red cells (10 ml), and PBS and 10% sucrose (10 ml). Virus titers varied from a pre-exposure titer of 8.8 log 10 $TCID_{50}$/ml to a post-exposure titer of 8.3. Mosquitoes, 5-8 days old, were exposed to the blood meal in droplet form placed on cage netting for ca. 1½ hours. Thereafter engorged (3/4 to full) females were selected and incubated for 14 days (ca. 28°C and 75-100% RH) at which time they were head squashed on glass slides. The resultant smears were stained with specific YF conjugate and examined by immunofluorescence for the presence of YF viral antigen. The results are shown in the following table.

	Filial Generation						
	0	1	2	3	4	5	6
0 Survival rate (%)	27	75	92	75	90	95	78
No. infect./Exam.	14/23	5/60	23/119	30/123	20/89	19/60	25/84
Infection rate (%)	41	8	19	24	22	32	30

Females of the original E_0 generation (i.e., progeny of eggs from wild-caught females) had a poor survival rate over the 2-week incubation period, making for few specimens for testing; otherwise the 7 generations were fairly comparable. The results indicate a sharp drop in infectivity following colonization, however the subsequent rise in susceptibility suggests that this negative effect may not be real, i.e., colonization for 6 generations did not

markedly change the susceptibility of Ae. aegypti to YF virus infection. The study also disclosed great variation in infectivity results between the 5 cages of mosquitoes comprising each filial generation, suggesting the need for an adequate number of cages in future infectivity studies of a comparative nature.

Suitability of fresh yellow fever virus vs. frozen virus for oral infection studies with mosquitoes (R.B.Tesh, T.H.G.Aitken and L. Lorenz). Mosquito infection studies to compare blood meals composed of fresh (actively growing) yellow fever (YF) virus and frozen YF virus were undertaken with Aedes aegypti (Amphur F_{24} strain). The reason for this study stems from earlier investigations with dengue virus where it was shown that mosquito (Ae. aegypti) infection and transmission studies were enhanced if actively-growing virus were used for blood meal preparation instead of frozen virus (Miller, et al., 1982, AJTMH 31: 1232).

In the present experiment, YF virus (Asibi) was grown in C6/36 cells for 6 days when it was harvested. One half of the recovered virus suspension was frozen (-70°C) for ca. 2 hours, after which it was thawed and the two virus preparations (fresh and frozen) were offered to starved mosquitoes as a blood meal in the form of: virus (2.5 ml), washed human red cells (10 ml), and PBS and 10% sucrose (10 ml). The meals were applied to mosquito cage netting as droplets; virus titers were 8.5 log 10 $TCID_{50}$/ml (pre meal) and 9.3 (post). Mosquitoes were exposed to the meals for ca. 1½ hours after which engorged females (3/4-full) were selected and incubated for 14 days (ca. 24°C and 70-100% RH) when they were harvested, head squashed, stained with specific YF conjugate and examined by immunofluorescence for presence of virus antigen. The results indicated that 40% of frozen meal mosquitoes were YF positive - 50/124 and 34% of the fresh virus mosquitoes were YF positive - 30/88. Thus, it would appear that frozen YF virus is equally efficient as actively growing YF virus as a source of virus for mosquito infection studies. As in the case of the "mosquito colonization" studies, these experiments disclosed a fairly wide variation of infectivity among the 7 cages of mosquitoes representing the two samples of virus.

Detection of dengue viral antigen in infected cell culture fluids and in suckling mouse brain suspensions by the modified double antibody sandwich enzyme linked immunosorbent assay (J.G. Olson and T.L. Thirkill). An antigen detection enzyme linked immunosorbent assay (ELISA) for dengue virus (DEN) was developed for virus stocks prepared in Aedes albopictus clone C6-36 cells and in suckling mouse brains. A pool of human convalescent sera from laboratory confirmed dengue fever patients was coated on 96-well flat bottomed microtiter plates. The virus stocks, previously titrated by either hemagglutination (HA) or by cell culture, and uninfected control antigens were added after the coating step. Mouse DEN immune ascitic fluids were added next followed by goat anti-mouse immunoglobulin conjugated with alkaline phosphatase. The enzyme substrate, p-nitrophenyl phosphate added in the final step, is colorimetrically altered in the presence of the bound enzyme. The subsequent reactions were read spectrophotometrically on a Titer-Tek Multiscan plate reader at 405 nm. Absorbance values exceeding the mean of multiple replicates of the uninfected control antigens plus three times their standard deviation were recorded as positive.

Using a combination of hyperimmune mouse ascitic fluids and mouse ascitic fluids which contained monoclonal DEN antibodies, we were able to detect and identify all 4 serotrypes of DEN. ELISA was a slightly less sensitive technique than cell culture (Table 34) and more sensitive than HA for detecting DEN antigen (Table 35). The ELISA was capable of detecting antigen at levels below those normally found in human patients with DEN infections (Table 36).

Preliminary attempts to detect antigen in patient sera were disappointing. Of 8 acute phase sera from dengue fever patients tested, only one gave a positive result. Similar negative results were observed when sera negative for DEN antibody by HI and NT testing were pooled and then used as a diluent for DEN antigen. This experiment suggested that the failure of the antigen detection ELISA to detect DEN antigen in human patient sera may have been due to a nonspecific interfering substance in serum. Two separate methods for treating serum were employed to remove substances which may be interfering with the test. Kaolin sulfate extracted and acetone extracted sera were used as diluent for DEN antigen with differing results. The interference observed with untreated sera continued after Kaolin treatment, but acetone extracted sera used as a diluent showed positive results similar to those using PBS-Tween as a diluent. Further tests are underway to determine whether DEN antigen can be detected in a dilution of antigen in serum followed by acetone extraction and in patient sera.

Table 34

Aedes albopictus clone C6/36 infected cell culture fluids

Virus	plaque forming units/ml	tissue culture infectious dose (50%)/ml		ELISA colorimetric 50% endpoint
		immuno fluorescence	cytopathic effect	
DEN-1	1.08×10^4	$10^{2.5}$	$10^{2.65}$	$10^{2.5}$
DEN-2	6.37×10^4	$10^{3.5}$	$10^{7.1}$	$10^{3.5}$
DEN-3	6.0×10^3	$10^{1.5}$	$10^{2.65}$	$10^{2.5}$
DEN-4	2.12×10^5	$10^{4.5}$	$10^{4.35}$	$10^{2.5}$

Table 35

Sucrose acetone extracted suckling mouse brain antigens

Virus	hemagglutination test		enzyme linked immunosorbent assay	
	titers*	minimum protein detected (g/ml)	titers*	minimum protein detected (g/ml)
dengue 1	64	68	10^2	44
dengue 2	256	19	10^3	50
dengue 3	256	49	10^2	124
dengue 4	64	135	10^3	86
Sindbis	256	49	ND**	125

*Reciprocal of highest dilution of antigen which gave a positive result.
**Not detected.

Table 36

Limits of dengue virus detected by enzyme-linked immunosorbent assay

Virus	plaque forming units/ml	minimum protein detected (g/ml)
DEN-1	3.29	12
DEN-2	2.52	11
DEN-3	2.45	35
DEN-4	14.6	9

Original antigenic sin in dengue fever patients (J. G. Olson). Studies by Okuno, et al (1980) and by Halstead (in press) have shown that dengue hemorrhagic fever (DHF) patients experiencing secondary infections with dengue (DEN) virus may respond immunologically with serum neutralizing antibodies directed against the DEN serotype which was responsible for the original infection and not the currently infecting strain. This phenomenon, known as original antigenic sin, is well described for influenza viral infections (Francis et al., 1953; and Davenport et al., 1953). The possibility that the host's response to a non-infecting serotype may help to explain why persons experiencing secondary DEN infections are more likely to develop DHF and dengue shock syndrome (DSS) was suggested by Okuno et al. (1980).

Our studies were designed to determine whether patients with uncomplicated dengue fever (DF) show evidence of original antigenic sin. The study population was comprised of 10 DF patients from whom virus was isolated and identified. Acute and convalescent phase sera were tested by hemagglutination inhibition (HI), indirect immunofluorescence (IFA) and microneutralization (NT) for antibodies. Results are summarized in table 37.

From the data in the table we classified 6 of the infections as primary and 4 as secondary. Secondary infections had evidence of DEN antibody on the acute phase when tested by IFA or NT. Among the patients who were experiencing their first infections with DEN virus, convalescent serum NT antibody titers were higher to a serotype other than the one isolated in all 6 (100%). Three of the 4 (75%) patients with secondary infections had higher NT antibody titers to a serotype other than the infecting one.

IFA test results were less easy to interpret since there was less variation in the antibody titers. However, in the patients experiencing their first DEN infections (14759 and 14760) the antibody titer to the infecting serotype was significantly lower than the others.

Clearly DF patients show evidence of original antigenic sin and this as an explanation for contributing to serious forms of DEN infections must be questioned. The observation of original antigenic sin in patients having their inital DEN infection suggests that our method of classifying the primary and secondary cases may have been incorrect. Other means of dichotomizing the population are being investigated.

Modified Southern blotting to determine genetic relatedness of RNA viruses (D.L. Knudson). A micro-extraction for dsRNA was developed (see 1981 Annual Report) that allows the preparation and processing of a large number of orbivirus isolates. Over 150 samples can be processed within 2 weeks including PAGE analysis. A second new technique has now been developed for dsRNA. The dsRNA is denatured by glyoxalation, and the ssRNA is separated by electrophoresis in agarose gels. The gel is treated following the Southern blotting protocol with modifications resulting in the transferance of ssRNA to nitrocellulose The RNA is bound to the sheet which is incubated in a solution of radioactive nucleic acid probe. Thus the genetic relatedness, determined by hybridization, can be shown. Since a by-product of the micro-extraction procedure is mRNA, the technology should be applicable also to ssRNA viruses, end-labeling the 3' end using the pCp technique representing the radioactive probe.

Table 37

IFA and neutralizing dengue antibody in patients following dengue viremia

Serum		virus isolated	days after onset	HI titer (JE)	IFA titer DEN-1	IFA titer DEN-2	IFA titer DEN-3	IFA titer DEN-4	NT titer DEN-1	NT titer DEN-2	NT titer DEN-3	NT titer DEN-4
13928	A	DEN-1	2	40	<5	20	40	<5	<4	<5	<10	<10
	C		12	>640	5,120	>20,480	5,120	>20,480	<4	320	80	>1280
14185	A	DEN-1	3	10	<5	<5	<5	<5	-	<5	<10	<10
	C		19	>640	5,120	10,240	5,120	5,120	16	80	40	160
14367	A	DEN-1	3	10	<5	<5	<5	<5	<4	<5	-	80
	C		10	>640	160	320	160	320	16	10	-	640
14759	A	DEN-1	1	10	<5	<5	<5	<5	<4	<5	<10	<10
	C		14	>640	40	2,560	640	5,120	16	40	40	320
14760	A	DEN-1	0	<5	<5	<5	<5	<5	<4	<5	<10	<10
	C		23	>640	320	2,560	1,200	2,560	32	80	160	640
15045	A	DEN-3	0	20	<5	5	20	10	<4	16	<4	<4
	C		21	320	2,560	5,120	2,560	5,120	128	2,560	2,560	256
15296	A	DEN-1	1	10	<5	<5	<5	<5	<4	<4	<4	<4
	C		11	160	1,280	2,560	5,120	5,120	<4	-	<4	<4
15316	A	DEN-2	2	<5	< 5	<5	<5	<5	8	<4	<4	<4
	C		13	>640	2,560	2,560	2,560	2,560	-	-	<4	<4
15353	A	DEN-3	2	10	5	5	<5	<5	<4	<4	<4	<4
	C		23	>640	2,560	10,240	10,240	20,480	<4	-	<4	<4
16012	A	DEN-3	1	10	5	<5	<5	<5	<4	<4	<4	<4
	C		12	320	2,560	5,120	5,120	5,120	<4	640	64	8

Detection of eastern equine encephalitis virus and Highland J virus antigens within mosquito pools by enzyme immunoassays (EIA) (S.W. Hildreth, B.J. Beaty, H.K. Maxfield, R.F. Gilfillan, and B.J. Rosenau). Enzyme-linked immunoassays were developed to replace common virological isolation and identification assays used in surveillance of arboviruses. These techniques were capable of detection and identification of EEE and HJ viruses within 24 hours after collection.

An indirect sandwich ELISA was used. Micro-titer plates were coated with anti-virus mouse IgG, followed by the sequential addition of mosquito pool sample, anti-virus rabbit IgG, goat anti-rabbit IgG conjugated with alkaline phosphatase, and substrate (either chromogenic= p-nitrophenyl phosphate or fluorogenic= 4-methyl umbelliferyl phosphate).

In laboratory studies, the minimum number of EEE or HJ infected Ae. triseriatus mosquitoes was determined using pools of known composition. Complete concordance was found between virus isolation with BHK-21 cells and the EIA with mosquito pools containing various numbers of non-infected mosquitoes (7 to 99) and 1 or 3 infected mosquitoes (all at 15 days post inoculation). Growth curve studies of EEE in parenterally infected Ae. triseriatus revealed that virus isolation was more sensitive than EIA (95.5% and 36.4%, respectively) during the first 3 extrinsic incubation days. After 3 days, however, there was complete concordance between the two assays; both techniques detected 100% of the infected pools. Specificity was 100% by both assays; serological cross-reactivity between EEE and HJ was minimal in the EIA.

In a retrospective study, the EIA was used to re-test 495 mosquito pools collected by the Massachusetts Department of Public Health (MDPH) between 1978 through 1981. These pools were tested for virus with a chick embryo tissue culture method. Of the 495 pools, 215 (43.4%) were diagnosed by MDPH to contain either EEE, HJ, or both viruses. Of these, 165/215 (76.7%) were found positive when tested by EIA. When the 50 "false negatives" were retested, virus was isolated from only 8 pools. Thus, an adjusted estimate of the sensitiveity of EIA was approximately 95%. Of the mosquito pools classified as positive by both assays, there was 98.7% agreement (kappa=0.987) in the identification of the two viruses. In one pool previously classified only as a EEE positive by MDPH, the EIA also detected HJ antigen. Of the 280 pools clasified by MDPH as negative, 278 (99.3%) were also negative by EIA, but 2 (0.7%) were positive. HJ virus was subsequently isolated from these two "false positive" pools.

For the purpose of surveillance of a known virus within a mosquito population, the EIA method for the detection of viral antigens appears to be as sensitive and specific as a standard surveillance method.

Field studies were initiated during 1981 and 1982 in Connecticut, New Jersey and Florida in order to obtain fresh materials for field-testing of the antigen-detection EIA and other newer technologies. These collections were funded by sources other than the Reference Center, but the surveillance results are reported here as background for the development of techniques which is currently in progress.

Field Studies in Connecticut (Ling Lee, L. Lorenz, T. Scott, M. Fletcher and A. J. Main): A limited field study was conducted at one locality in southern Connecticut during the summer and fall of 1982. One purpose of the project was to collect louse-flies (Hippoboscidae) for colonization and vector competency studies in the laboratory. A small number of Ornithomya anchineuria (6 males, 11 females) and Ornithoica vicina (2 females) were collected from 307 birds examined at the Hammonasset River Study Site in Killingworth, Connecticut (Table 36). These were supplemented with 53 males and 51 females O. anchineuria and 3 female O. vicina collected by Dr. Trevor Lloyd-Evans at the Manomet (Massachusetts) Bird Observatory; however, attempts to rear them on house sparrows were unsuccessful.

Ixodes dammini ticks were collected from 7% of the birds and 64% of the mammals examined at this site (Table 38). One nymphal Haemaphysalis leporispalustris was removed from a rufous-sided towhee and 15 Dermacentor variabilis were taken from 6 mammals (Table 38). Previous studies in this area yielded fewer I. dammini and more D. variabilis, confirming our previous observations of the spread and increased populations of I. dammini.

Blood samples were drawn from 180 of the 307 birds and 53 of the 56 mammals captured at the study site. While virus was not detected in any of these samples using Vero cells, these samples await EIA antigen detection. Serologic evidence suggests that eastern equine encephalomyelitis virus was circulating in the area. EEE HI antibody was demonstrated in 8% of the avian sera and 6% of the mammalian sera (Table 39) including at least three first year birds indicating current activity (Table 40). Antibody was also detected in three blood samples from two immature white-footed mice suggesting spillover into the mammalian populations. Highlands J antibody was demonstrated in 2% of the birds. All these seropositive birds were at least one-year-old, making it impossible to pinpoint the time and place of infection. Antibody was detected in one sample using a WEE antigen, although this same sample had a higher titer to Highlands J virus (1:20 and 1:40, respectively). SLE antibody was not demonstrated in any of the bird sera and only one mammal sample reacted with the SLE antigen (1:10). This same sample had a higher (1:160) titer to Powassan virus. Both opossums sampled had POW antibody.

Twenty species of mosquitoes were collected in CDC light traps at the Hammonasset River Study Site (Table 41). All 1,244 adult female mosquitoes were tested in suckling mice; virus was not recovered from any of the pools. These pools well be tested by EIA for antigen detection. Coquillettidia perturbans, Culiseta melanura, and Aedes canadensis were the three most abundant species, accounting for 70% of the collections. Fifty additional female mosquitoes, including 31 Cs. melanura, 11 Cs. morsitans, 7 Anopheles punctipennis, and 1 Culex restuans, were aspirated from natural shelters in Farmington, Connecticut. All were negative in suckling mice for virus isolation attempts.

Table 38

Ticks and louse-flies collected from birds and mammals at the Hammonasset River Study Site in Killingworth, Connecticut during 1982

	No. Examined	Ticks *I. dammini*			Louse-flies *O. anchineuria*	Louse-flies *O. vicina*
		Larvae	Nymphs	Adults	Adults	Adults
Downy Woodpecker	8					
Blue Jay	2	4/4*				
Black-capped Chickadee	13					
Gray Catbird	33		1/1		1/1	
Wood Thrush	23		2/2		9/4	1/1
Hermit Thrush	10					
Veery	7	1/1	7/3		1/1	1/1
Yellow-throated Vireo	1		9/1			
Black & White Warbler	11		3/1			
Yellow-rumped Warbler	38					
Ovenbird	24		2/1		3/1	
Common Yellowthroat	12		2/2			
Canada Warbler	8					
American Redstart	10		1/1		1/1	
Scarlet Tanager	4		1/1			
Cardinal	8	2/2	19/3		1/1	
Rufous-sided Towhee**	15	11/5	4/2		1/1	
White-throated Sparrow	16					
Song Sparrow	19		5/3			
Other Birds(18 species)	45					
Total Birds	307	18/19	56/21	-	17/10	2/2
Opossum	2		4/2			
Short-tailed Shrew	3					
White-footed Mouse**	35	121/27	5/5			
Meadow Jumping Mouse	8	3/1				
Norway Rat**	1		2/1			
Raccoon**	6	1/1	8/3			
Gray Fox	1					
Man**	(3)		1/1	2/2		
Total Mammals	58	125/29	20/12	2/2	-	-

*Number of ectoparasites/number of hosts.

**Plus 1 nymphal *Haemaphysalis leporispalustris* from a towhee and 15 *Dermacentor variabilis* from two white-footed mice (1L, 1N), a Norway rat (1L), 2 raccoons (6 males, 5 females), and man (1 female).

Table 39

Virus and antibody in birds and mammals from the Hammonasset River Study Site during 1982

		HI Antibody		
	No. Tested	EEE	HJ	POW
Summer Resident Species				
Eastern Phoebe	3			
Gray Catbird	33	6	2	0
American Robin	3			
Wood Thrush	20	2		
Hermit Thrush	10			
Veery	7			
Ovenbird	7			
Northern Waterthrush	2			
Scarlet Tonager	4			
Rose-breasted Grosbeak	1			
Rufous-sided Towhee	14	1		
Song Sparrow	18			
Swamp Saprrow	2			
Subtotals	124	9	2	0
Permanent Resident Species				
Hairy Woodpecker	4		1	
Downy Woodpecker	7			
Blue Jay	2			
Black-capped Chickadee	11	1	0	
Tufted Titmouse	4			
Cardinal	8	1		
Subtotals	36	2	1	0
Migrants & Winter Resident Species				
Swainson's Thrush	4			
Slate-colored Junco	1			
White-throated Sparrow	15	4	0	
Subtotals	20	4	0	0
Total Birds	180	15	3	0
Mammals				
Opossum	2			2
Short-tailed Shrew	1			
White-footed Mouse	35	3		
Meadow Jumping Mouse	7			
Norway Rat	1			
Raccoon	6			
Gray Fox	1			
Total Mammals	53	3	0	2

*all samples were negative for virus in vero cells and for WEE (except one Gray Catbird with a higher titer to HJ) and SLE (except one opossum with a higher titer to POW) HI antibody.

Table 40

Seropositive samples from birds and mammals at the Hammonasset River Study Site during 1982

Blood No.	Species	Age	Sex	Date	EEE	HJ	WEE	POW	SLE
B-16-82	Wood Thrush	AHY	F	7/13/82	1:160	-	-	-	-
B-24-82	Gray Catbird	AHY	F	7/14/82	1:10	-	-	-	-
B-28-82	Hairy Woodpecker	AHY	F	7/21/82	-	1:20	-	-	-
B-43-82	Rufous-sided Towhee	AHY	F	7/22/82	1:160	-	-	-	-
B-50-82	Gray Catbird	AHY	F	7/28/82	-	1:10	-	-	-
B-90-82	Gray Catbird	AHY	M	8/19/82	1:20	-	-	-	-
B-118-82	Song Sparrow	AHY	M	9/04/82	1:40	-	-	-	-
B-119-82	Cardinal	AHY	F	9/04/82	1:20	-	-	-	-
B-128-82	Gray Catbird	AHY	?	9/04/82	-	1:40	1:20	-	-
B-129-82	Gray Catbird	AHY	?	9/04/82	1:80	-	-	-	-
B-145-82	White-throated Sparrow	?	?	10/08/82	1:40	-	-	-	-
B-158-82	Gray Catbird	HY	?	10/09/82	1:10	-	-	-	-
B-159-82	Gray Catbird	HY	?	10/09/82	1:80	-	-	-	-
B-165-82	Wood Thrush	HY	?	10/09/82	1:20	-	-	-	-
B-168-82	White-throated Sparrow	?	?	10/09/82	1:20	-	-	-	-
B-172-82	Black-capped Chickadee	AHY	?	10/15/82	1:20	-	-	-	-
B-173-82	White-throated Sparrow	?	?	10/15/82	1:20	-	-	-	-
B-174-82	White-throated Sparrow	?	?	10/15/82	1:20	-	-	-	-
M-13-82	Opossum	Imm	M	08/11/82	-	-	-	1:20	-
M-23-82	White-footed Mouse	Imm	M	08/25/82	1:80	-	-	-	-
M-29-82	White-footed Mouse	Imm	M	09/04/82	1:80	-	-	-	-
M-39-82	Opossum	Adult	F	10/09/82	-	-	-	1:160	1:10
M-45-82	White-footed Mouse	Imm	M	10/16/82	1:20	-	-	-	-

- = < 1:10 AHY = after hatching year HY = hatching year ? = unknown

F = Female M = Male Imm = Immature

M-23-82 and M-29-82 are from the same mouse.

Table 41

Adult female mosquitoes from the Hammonasset River Study Site tested for virus* during 1982

	July	August	September	October	Total
Anopheles crucians		1			1
Anopheles punctipennis	13	31	1	1	46
Anopheles quadrimaculatus	4				4
Anopheles walkeri				1	1
Aedes abserratus	4				4
Aedes aurifer	53	6			59
Aedes canadensis	99	23	1	2	125
Aedes cantator	1	2			3
Aedes cinereus	55	.6			61
Aedes excrucians group	14	3			17
Aedes thibaulti	4				4
Aedes triseriatus/hendersoni	5	6	3		14
Aedes vexans	30	7		4	41
Culex pipiens	5	5			10
Culex restuans	52	14		1	67
Culex salinarius	13	19		1	33
Culiseta melanura	72	111	3	4	190
Culiseta morsitans	4	4			8
Coquillettidia perturbans	285	263	5		553
Uranotaenia sapphirina		1		2	3
Total	713	502	13	16	1244
Trap Nights	12	14	4	6	36

*All samples were negative in suckling mice.

Arbovirus surveillance in New Jersey and Florida (Ling Lee, L. Lorenz, and A.J.Main). During 1982, a cooperative program on the epidemiology of EEE virus in New Jersey was initiated with Dr. Wayne Crans of Rutgers University. Blood samples from 1096 birds and 37 reptiles collected at two sites in 1981 and from 532 birds captured at one site in 1982 were submitted for virus isolation attempts in Vero cells and for HI and plaque-reduction neutralization tests for EEE, Highlands J, and SLE antibody (Table 42). EEE virus was recovered from 0.4% of the birds from the first year and from 1.2% the second; Highlands J virus was isolated from birds only once in 1981 and twice in 1982 (Table 43). Antigen detection EIA will be initiated on these samples. EEE HI antibody was detected in 7.9% of the birds from West Creek tested to date (Table 42) including 1.7% of the first year birds suggesting current virus activity in the area (Table 44). Less than half of the samples have been tested; those collected between mid-June and early September, remain. At Dennisville, where all of the HI testing for 1981 is complete, 28.8% of all birds and 28.9% of the hatching year birds have EEE antibody (Table 42). Highlands J antibody was detected in 10.7% and 12.4%, at the two sites, respectively, in all birds and 6.9% and 11.7% of the first year birds (Table 42). SLE antibody was not detected at either site. None of the sera from 1982 have been tested by HI yet.

EEE HI antibody was detected in one of 19 box turtles (5%) and 10 of 17 mud turtles (59%) collected in Dennisville during 1981 (Table 42, 45). Highlands J antibody was not detected in any of the reptiles and one serum from a mud turtle gave a 1:10 reaction with SLE antigen.

In addition to the bird and reptile bloods, 13,368 adult female mosquitoes were submitted for virus isolation attempts in suckling mice and for antigen-detection EIA. These included 12,606 Aedes sollicitans and 762 Coquillettidia perturbans and represent approximately one-third of the Ae. sollicitans collections in New Jersey during 1982 (Table 46). While EEE, Highlands J, and SLE viruses were not recovered from any of the 149 pools tested at YARU, 14 isolates of a Cache Valley-like virus were isolated from Ae. sollicitans collected at Dennisville (Table 47). This represents approximately 12% of the pools of Ae. sollicitans from this site for a minimum field infection ratio of 1:754. The remaining two-thirds of the Ae. sollicitans were tested in wet chicks at the New Jersey state virus laboratory as were all of the Culiseta melanura. EEE virus was recovered from three pools of Ae. sollicitans and many pools of Cs. melanura in that laboratory.

Approximately 6,150 Dermacentor variabilis and 1,096 Amblyomma maculatum collected from feral pigs in Florida were received from Dr. E.P.J. Gibbs of the University of Florida for virus isolation attempts in suckling mice. Virus was not recovered from 700 D. variabilis or 575 A. maculatum tested to date.

Table 42

Virus and antibody in birds and reptiles captured in New Jersey during 1981 and 1982

	West Creek			Dennisville			
	1981			1981			1982
	Virus	EEE	HJ	Virus	EEE	HJ	Virus
Yellow-billed Cuckoo				0/5	0/5	0/5	1/10
Common Flicker	0/4	0/2	1/2	0/8	0/8	4/8	0/5
Hairy Woodpecker	0/1	0/1	0/1	0/6	2/6	2/6	0/5
Downy Woodpecker	0/3	0/1	0/1	0/9	0/9	0/9	0/6
Great-crested Flycatcher				0/5	1/5	1/5	0/8
Blue Jay	0/5	2/4	2/4	0/17	11/17	11/17	0/13
Carolina Chickadee	0/14	0/6	0/6	0/80	29/80	9/80	1/48
Tufted Titmouse	0/11	0/4	1/4	1/39	16/39	12/39	1/26
Red-breasted Nuthatch				0/10	0/10	0/10	
Gray Catbird	1/126	5/33	8/33	0/50	13/50	17/50	1/40
American Robin	1/22	2/17	1/17	0/3	1/3	1/3	0/3
Wood Thrush	0/36	3/10	3/10	0/69	40/69	34/69	0/51
Swainson's Thrush	0/4	0/3	0/3	0/7	0/7	0/7	0/6
Veery	0/16	0/3	0/3	0/5	0/5	0/5	0/9
Ruby-crowned Kinglet				0/1	0/1	0/1	1/2
White-eyed Vireo	0/18	1/11	3/11	0/7	0/7	1/7	0/10
Red-eyed Vireo	0/24	0/10	0/10	0/13	2/13	4/13	0/2
Black & White Warbler	0/13	0/8	1/8	0/22	5/22	0/22	0/21
Black-throated Blue W.				0/9	3/9	0/9	0/5
Yellow-rumped Warbler	0/18	1/18	0/18	0/24	1/24	0/24	0/62
Pine Warbler				0/17	5/17	0/17	1/10
Ovenbird	0/14	0/5	0/5	1/64	24/64	8/64	0/60
Northern Waterthrush	0/11	0/5	0/5	0/5	0/5	0/5	0/8
Common Yellowthroat	0/28	0/15	0/15	1/14	4/14	2/14	0/23
American Redstart	0/11	0/6	0/6	0/8	0/8	1/8	0/14
Other Warblers	0/17	0/4	0/4	0/10	2/10	0/10	0/18
Redwinged Blackbird				0/5	0/5	0/5	1/3
Brown-headed Cowbird	0/4	0/3	0/3	0/6	1/6	0/6	0/3
Common Grackle	0/1			0/36	0/36	1/36	0/22
Cardinal	0/3	0/3	0/3	0/26	8/26	0/26	0/15
Indigo Bunting				0/12	1/12	0/12	0/3
Rufous-sided Towhee	0/26	2/13	1/13	0/3	1/3	0/3	
Slate-colored Junco	0/1	0/1	0/1				1/3
White-throated Sparrow	0/18	0/18	1/18	0/2	0/2	0/2	
Other birds	0/19	1/10	1/10	0/31	7/31	5/31	0/18
Total Birds	2/468	17/214	23/214	3/628	181/628	109/628	8/532
Percent positive	0.4	7.9	10.7	0.5	28.8	17.4	1.5
Box Turtle				0/19	1/19	0/19	
Mud Turtle				0/17	10/17	0/17	
Northern Water Snake				0/1	0/1	0/1	
Total Reptiles				0/37	11/37	0/37	
Percent Positive					29.7		

Table 43

Virus isolations from birds collected in New Jersey

Blood No.	Species	Age	Sex	Date	Locality	HI Antibody EEE	HI Antibody HJ
EEE Virus:							
DV-349 (1981)	Tufted Titmouse	HY	?	7/16/81	Dennisville	-	-
DV-465 (1981)	Ovenbird	HY	?	8/19/81	Dennisville	-	-
DV-556 (1981)	Common Yellowthroat	HY	M	9/09/81	Dennisville	1:80	-
WC-415 (1981)	American Robin	HY	?	9/21/81	West Creek	-	-
DV-50 (1982)	Pine Warbler	AHY	M	5/25/82	Dennisville	NT	NT
DV-52 (1982)	Yellow-billed Cucko	AHY	?	5/25/82	Dennisville	NT	NT
DV-60 (1982)	Carolina Chickadee	AHY	F	5/26/82	Dennisville	NT	NT
DV-86 (1982)	Gray Catbird	AHY	F	6/08/82	Dennisville	NT	NT
DV-89 (1982)	Red-winged Blkbird	AHY	F	6/08/82	Dennisville	NT	NT
DV-260 (1982)	Tuffed Titmouse	HY	?	7/27/82	Dennisville	NT	NT
Highlands J Virus:							
WC-431 (1981)	Gray Catbird	HY	?	9/30/81	West Creek	-	-
DV-447 (1982)	Ruby-crowned Kinglet	?	M	10/07/82	Dennisville	NT	NT
DV-448 (1982)	Slate-colored Junco	HY	?	10/07/82	Dennisville	NT	NT

- = <1:10 NT = Not Tested HY = hatching year AHY = after hatching year
? = unknown M = Male F = Female

Table 44

Virus and antibody in first year (hatching year) birds captured in New Jersey during 1981 and 1982

	West Creek 1981			Dennisville 1981			1982
		Antibody			Antibody		Antibody
	Virus	EEE	HJ	Virus	EEE	HJ	Virus
Downy Woodpecker				0/7			0/3
Blue Jay	0/1			0/7	3/7	3/7	0/8
Carolina Chickadee	0/3	0/3	0/3	0/26	11/26	2/26	0/21
Tufted Titmouse	0/7	0/2	0/2	1/25	10/25	5/25	1/22
Gray Catbird	1/39	0/9	2/9	0/6	2/6	2/6	0/9
American Robin	1/3	0/3	1/3.	0/1	0/1	0/1	0/1
Wood Trhush	0/11			0/14	8/14	4/14	0/20
Swainson's Thrush	0/3	0/3	0/3	0/6	0/6	0/6	0/6
Veery	0/9	0/1	0/1	0/3	0/3	0/3	0/8
White-eyed Vireo	0/2			0/3	0/3	1/3	0/3
Red-eyed Vireo	0/14	0/6	0/6	0/3	0/3	1/3	0/1
Black & White Warbler	0/1			0/9	2/9	0/9	0/15
Black-throated Blue W.				0/6	2/6	0/6	0/5
Yellow-rumped Warbler	0/17	1/17	1/17	0/19	0/19	0/19	0/61
Pine Warlber				0/4	1/4	0/4	0/3
Ovenbird	0/2	0/1	0/1	1/38	16/38	7/38	0/41
Northern Waterthrush	0/5	0/1	0/1	0/3	0/3	0/3	0/8
Common Yellowthroat	0/2			1/7	4/7	2/7	0/10
American Redstart	0/6	0/2	0/2	0/1	0/1	0/1	0/9
Other Warblers	0/3						0/10
Common Grackle				0/20	2/20	0/20	0/5
Cardinal				0/18	8/18	0/18	0/9
Rufous-sided Towhee	0/5	0/2	0/2				
Slate-colored Junco							1/3
White-throated Sparrow	0/7	0/7	1/7				
Other Birds	0/2	0/1	0/1	0/13	0/13	0/13	0/19
Total Birds	2/142	1/58	4/58	3/239	69/239	28/239	2/300
Percent Positive	1.4	1.7	6.9	1.3	28.9	11.7	0.7

Table 45

Hemagglutination-inhibiting antibody in turtles collected in Dennisville, New Jersey, during 1981

Blood No.	Species	Age	Sex	Date	HI Antibody EEE	HJ	SLE
DV- 74	Box Turtle	Adult	M	5/13/81	1:20	-	-
DV-155	Mud Turtle	?	?	5/27/81	1:20	-	-
DV-161	Mud Turtle	?	?	5/27/81	1:20	-	-
DV-162	Mud Turtle	?	?	5/27/81	1:20	-	-
DV-178	Mud Turtle	Adult	F	5/28/81	1:20	-	-
DV-184	Mud Turtle	Adult	F	5/28/81	1:40	-	-
DV-185	Mud Turtle	Adult	F	5/28/81	1:40	-	-
DV-201	Mud Turtle	Adult	F	6/02/81	1:80	-	1:10
DV-202	Mud Turtle	Adult	F	6/02/81	1:160	-	-
DV-255	Mud Turtle	?	F	6/24/81	1:80	-	-
DV-281	Mud Turtle	?	F	7/01/81	1:320	-	-

Table 46

Virus isolation attempts from Aedes sollicitans and Coquillettidia perturbans collected in New Jersey during 1982

	No. Tested	No. Pools tested	No. Pools negative	CV-like isolates
Aedes sollicitans				
Dennisville	10,562	118	104	14
Leed's Point	2,017	20	20	0
Stone Harbor	27	1	1	0
Total	12,606	139	125	14
Coquillettidia perturbans				
Dennisville	87	2	2	0
Franklinville	200	2	2	0
Iona Lake	75	2	2	0
Pond Creek	400	4	4	0
Total	762	10	10	0

Table 47

Cache Valley-like virus isolates from Aedes sollicitans collected at Dennisville, New Jersey, during 1982

Strain	Pool size*	Date Collected
RU- 68	100	9/13/82
RU- 69	100	9/13/82
RU- 70	100	9/13/82
RU- 75	100	9/16/82
RU- 92	38	9/21/82
RU-100	100	9/30/82
RU-101	100	9/30/82
RU-102	100	9/30/82
RU-107	100.	10/01/82
RU-109	100	10/01/82
RU-110	100	10/01/82
RU-111	100	10/01/82
RU-114	100	10/01/82
RU-139	100	10/07/82

*All were nonblooded adult female Ae. sollicitans

VI. COLLECTION OF LOW PASSAGE VIRUS REFERENCE STRAINS (R.B. Tesh and A.J. Main)

As noted in our last annual report, an effort has been made to collect and to prepare lyophilized stocks of low passage strains of arboviruses of public health and veterinary importance. Last year we reported strains of yellow fever and chikungunya viruses, which were prepared in 1980-81. During the current year, stocks of an additional 52 virus strains were prepared and lyophilized. These agents as well as their origin and passage history are listed in Table 48. Letters of inquiry have recently been sent to a number of arbovirus laboratories, requesting other samples of low passage virus strains from different geographic localities and time periods.

We intend to create a data file with pertinent information on each virus in the collection. This information as well as the lyophilized virus stocks will be available to interested investigators at no cost. It is anticipated that this collection will prove to be an invaluable reference resource for future comparative studies of viral genetics, biochemistry, pathogenicity and antigenic relationships.

Table 48

Low-Passage Virus Strains Obtained and Lyophilized 1982-1983

Virus Identification	Genus	Strain	Passage History	Source	Locality	Date
Highlands J	Alphavirus	78-3331	C6/36 #1	Culiseta melanura	Canton, Mass.	Sept.
Highlands J	Alphavirus	79-2137	C6/36 #1	" "	Westport,Mass.	Aug.
EEE	Alphavirus	78-3372	C6/36 #1	" "	Raynham,Mass.	Sept.
EEE	Alphavirus	79-2138	C6/36 #1	" "	Westport,Mass.	Aug.
Chagres	Phlebovirus	PaAr3419	Vero #2	Lutzomyia sanguinaria	Bayano, Panama	Oct.
Punta Toro	Phlebovirus	PaAr 2381	Vero #2	" "	Bayano, Panama	Nov.
DEN-1	Flavivirus	Fiji 40130	Mosq ?,C6/36 #1	Human serum	Fiji	
DEN-1	"	Manila 19078	" "	" "	Manila,Philippines	
DEN-1	"	Burma 10378	" "	" "	Burma	
DEN-1	"	Jamaica 44684	" "	" "	Jamaica	
DEN-1	"	Nauru 16299	" "	" "	Nauru	
DEN-1	"	Dak H29177	Mosq #1,C6/36#1	" "	Bandia,Senegl	
DEN-2	"	Bangkok 16803	Mosq ? ,C6/36#1	" "	Bangkok,Thailand	
DEN-2	"	Indonesia 10410	" "	" "	Java, Indonesia	
DEN-2	"	JA-TVP-496	C6/36 #1	" "	Jamaica	Aug.
DEN-2	"	NC 9163	" "	" "	New Caledonia	
DEN-2	"	Burma 40479	" "	" "	Burma	
DEN-2	"	PM 33974	Mosq #1,C6/36#1	Aedes africanus	Rep. of Guinea	Nov.
DEN-2	"	PR-159	C6/36 #1	Human serum	Puerto Rico	
DEN-3	"	Burma DHF 190	Mosq ?, C6/36#1	" "	Burma	
DEN-3	"	Tahiti 18	" "	" "	Tahiti	
DEN-3	"	PR-9311	" "	" "	Puerto Rico	
DEN-3	"	Thailand 49080	" "	" "	Thailand	
DEN-3	"	Singapore 16182	" "	" "	Singapore	
DEN-4	"	Medan 12524	" "	" "	Sumatra,Indonesia	
DEN-4	"	Tahiti 79	" "	" "	Tahiti	
DEN-4	"	Sri Lanka	" "	" "	Sri Lanka	
DEN-4	"	Niue	" "	" "	Niue	
DEN-4	"	Gilberts 49367	" "	" "	Kiribati,Gilberts	
DEN-4	"	PR-TVP 376	C6/36 #1	" "	Puerto Rico	Feb.
Snowshoe hare	Bunyavirus	82-Y-21	C6/36 #1	Aedes nigripes	Yukon Ter.,Canada	July

Table 48 continued

Virus Identification	Genus	Strain	Passage History	Source	Locality	Date
JE	Flavivirus	Osaka 222681	Mosq ?,C6/36#1	Culex tritaeniorhynchus	Osaka,Japan	
JE	"	Sagiyama 224052	" "	" "	Sagiyama, Japan	
JE	"	Java 222682	" "	" "	Java, Indonesia	
JE	"	HK 8256	Mosq #7,C6/36#1	Culex annulus	Taiwan	
YF	"	M 37	Mosq #2,C6/36#1	Human serum	Senegal	Nov.
YF	"	PM 27340	Mosq #2,C6/36#1	Aedes furcifer-taylori	Kedougou,Senegal	Oct.
Karimabad	Phlebovirus	91045-AG	Vero #3	Phlebotomus papatasi	Isfahan,Iran	Aug.
Sicilian	Phlebovirus	91025-B	Vero #3	" "	Isfahan, Iran	
Isfahan	Vesiculovirus	91025-C	Vero #2	" "	Isfahan, Iran	Aug.
Isfahan	"	91026-167	Vero #3	" "	Isfahan, Iran	Aug.
Alajuelva	Bunyavirus	76V-2441	Mosq #1,C6/36#1	Aedeomyia squamipennis	Vinces, Ecaudor	May
Kokobera	Flavivirus	CH 19620	Mosq #2,C6/36#1	Culex annulirostris	Charleville,Aust.	Feb.
Keystone	Bunyavirus	MB7-34EJ	Mosq #1,C6/36#1	Aedes atlanticus-tormentus	Bay St.Louis,Miss.	Sept.
San Angelo	Bunyavirus	72V-4089	Mosq #2,C6/36#1	Psorophora signipennis	Las Cruces, N.M.	
Cache Valley	Bunyavirus	69V-2152	C6/36 #1	Culiseta inornata	Umatilla Co.,Oregon	
EEE	Alphavirus	ME-77132	Mosq #1,C6/36#1	Culiseta melanura	Carver Cdr Swmp,Ma.	Aug.
Ross River	Alphavirus	S-48325	C6/36 #4	Human serum	Pago Pago,Am Samoa,	Dec.
Kunjin	Flavivirus	CH-16532	C6/36 #2	Culex annulirostris	Charleville,Aust.,	March
SLE	Flavivirus	WR-Ft.Wash.4	Mosq #2,C6/36#2	Culex pipiens	Ft.Washington,MD.	Jan.
CHIK	Alphavirus	1455/75	Mosq #2,C6/36#1	Human serum	Bangkok,Thailand	
CHIK	Alphavirus	PO-731460	Vero #1,Mosq #1	Human serum	Barsi, India	

VII. DISTRIBUTION OF REAGENTS, WHO COLLABORATING CENTRE FOR REFERENCE AND RESEARCH (R.E. Shope, A. Main, R. Tesh, S. Buckley, G.H. Tignor, J. Meegan).

The equivalent of 568 ampoules of arbovirus reagents were distributed from the WHO Centre to laboratories in 23 countries during 1982. This total consisted of 206 ampoules of virus stock, 237 ampoules of virus antigen, and 125 ampoules of mouse immune ascitic fluid or sera. Of the viruses and antibody distributed, there were represented 110 different arboviruses.

During 1982, the equivalent of 245 ampoules of arbovirus reagents was referred to this Centre from laboratories in 13 different countries. The referrals consisted of 176 viruses (Table 49), 3 ampoules of antigens, and 66 ampoules of immune reagents. In addition, 723 human, 157 ungulate, 1279 wild bird, and 80 wild rodent sera were received for arbovirus antibody survey testing and 37 human sera for diagnostic tests.

Seven different cell lines were distributed in 1982. The lines and recipients are listed in Table 50.

Table 49

Virus referred to YARU for identification and study, 1982

Country of origin; strain	Source	Information from donor	YARU identification
Australia			
OR 189	*Culex annulirostris*	new rhabdovirus, Parry Creek	new rhabdovirus
OR 194	*Aedomyia catastica*	new rhabdovirus, Kununurra	new rhabdovirus
OR 250	*Culex annulirostris*	new rhabdovirus, Kimberley	new rhabdovirus
Aus 96037		new virus	
Brazil			
SPAn 64962		Cananeia, new virus	
Burma			
Bu 40546	human serum	dengue, type 1	
Bu 40979	human serum	dengue, type 2	
Bu DHF190	human serum	dengue, type 3	
Canada			
Ca 1216-77	*Aedes vexans*	Cache Valley	
Ca 898-77	*Culiseta morsitans*	Cache Valley	
Ca 943-77	*Culiseta inornata*	Cache Valley	
Ca 550-81	*Culiseta inornata*	Cache Valley	
Ca 629-81	*Culiseta inornata*	Cache Valley	
Ca 82Y21	*Aedes nigripes*	snowshoe hare	
Ca 82Y46	*Aedes nigripes*	snowshoe hare	
Ca 82Y69	*Aedes communis*	snowshoe hare	
Ca 82Y188	*Aedes hexodontis*	snowshoe hare	

Table 49 (continued)

Virus referred to YARU for identification and study, 1982

Country of origin; strain	Source	Information from donor	YARU identification
Central African Republic			
Dak AnB 1227			
Dak ArB 2078	Culex perfuscus	Orungo virus	
Dak ArB 3689		new virus, Bambari	
Ecuador			
76V1565		Palestina, new virus	
76V4357		VSV-New Jersey	
75V3066		Playas, new virus	
75V807		Vinces, new virus	
77V14814		Brucouna, new virus	
Egypt			
EgAn 1245	rodent	phlebotomus fever group	
EgAn 1270	rodent	phlebotomus fever group	
EgAn 1273	rodent	phlebotomus fever group	
EgAn 1291	rodent	phlebotomus fever group	
Ethiopia			
OMR virus	rodent	new virus	
Fiji			
Fi 40130	human serum	dengue, type 1	

Table 49 (continued)

Virus referred to YARU for identification and study, 1982

Country of origin; strain	Source	Information from donor	YARU identification
France			
FrT 439	tick		
FrBrestArT 707	tick		
FrBrestArT 261	tick		
Gilberts			
Gi 49376	human serum	dengue, type 4	
Greece			
Ap-92			
GrPaAr 814	Phlebotomus	unknown virus	Phlebotomus fever gp.
Vergina virus	ticks	new subtype of CETB	
Indonesia			
Indo 222682	Culex tritaeniorhynchus	Japanese encephalitis	
Indo 10410	human serum	dengue, type 2	
Indo Medan 12524	human serum	dengue, type 4	
Indo 9340/6468	Culex tritaeniorhynchus	unknown virus	group B
Indo 9338/6466	Culex tritaeniorhynchus	unknown virus	
Indo 9374/6502	Culex tritaeniorhynchus	unknown virus	
Indo 9384/6512	Anopheles subpictus	unknown virus	
Indo 9411/6539	Culex tritaeniorhynchus	unknown virus	
Indo 7694/5225	Anopheles vagus	unknown virus	
Indo 7910/5441	Anopheles vagus	unknown virus	group B
Indo 8923/8106	Culex tritaeniorhynchus	unknown virus	
Indo 8935/8118	Culex fuscocephalus	unknown virus	
Indo 8949/8132	Culex vishnui	unknown virus	

Table 49 (continued)

Virus referred to YARU for identification and study, 1982

Country of origin; strain	Source	Information from donor	YARU identification
Indonesia continued			
Indo 9219/8442	Culex tritaeniorhynchus	unknown virus	group B
Indo 10817/8547	Anopheles annuloris	unknown virus	
Indo 10915/8645	Culex vishnui	unknown virus	
Indo 10920/8650	Anopheles vagus	unknown virus	
Indo 11376/9126	Anopheles vagus	unknown virus	
Ireland			
59972-6		Soldado Rock, Hughes gp.	
Italy			
ISS Phl 18	Phlebotomus	unknown virus	
Ivory Coast			
DakArA 142/79	Aedes taylori	Orungo virus	
DakArA 1131	Culex abserratus	Unknown virus	
Dak ArA3673	Culex group rima	unknown virus, Olifantsvlei gp.	
Jaimaica			
Jam 44684	human serum	dengue, type 1	
Japan			
Ja 222681	Culex tritaeniorhynchus	Japanese encephalitis	
Ja 224052	Culex tretaeniorhynchus	Japanese encephalitis	
Cap 44		Hirota virus, Nyamanini gp.	

Table 49 (continued)

Virus referred to YARU for identification and study, 1982

Country of origin; strain	Source	Information from donor	YARU identification
Philippines			
Ma 19076	human serum	dengue, type 1	
Nauru			
Na 16299	human serum	dengue, type 1	
New Caledonia			
NC 9163	human serum	dengue, type 2	
New Zealand			
NZ T1	*Ornithodoros capensis*	Hughes group	
NZ T497	*Ornithodoros capensis*	Hughes group	
NZ T26	*Ornithodoros capensis*	Hughes group	
NZ T414	*Ornithodoros capensis*	Hughes group	
NZ T469	*Ornithodoros capensis*	Hughes group	
NZ T58	*Ornithodoros capensis*	Johnston Atoll	
NZ T580	*Ixodes eudyptidis*	Saumarez Reef	
Niue			
Ni 49490	human serum	dengue, type 4	

Table 49 (continued)

Virus referred to YARU for identification and study, 1982

Country of origin; strain	Source	Information from donor	YARU identification
Panama			
PaAr 2381	*Lutzomyia sanguinaria*	Punta Toro	
PaAr3419	*Lutzomyia sanguinaria*	Chagres	
Pa903681	*Choleopus hoffmanni*	Changuinola group	
Pa903684	*Choleopus hoffmanni*	Changuinola group	
Pa903682	*Choleopus hoffmanni*	Changuinola group	
Pa903683	*Choleopus hoffmanni*	Changuinola group	
Pa903686	*Choleopus hoffmanni*	Changuinola group	
Pa903685	*Choleopus hoffmanni*	Changuinola group	
Pa401247	*Choleopus hoffmanni*	Unknown	
Puerto Rico			
PR 9311	human serum	dengue, type 3	
Senegal			
PM 27340/M14	*Aedes furcifer-taylori*	yellow fever	
M 37	human serum	yellow fever	
Dak 1279		yellow fever	
SH 29177/M28	human serum	dengue, type 1	
PM 33974/M33	*Aedes africanus*		dengue, type 2
Dak HD 22196	human serum	Orungo	
Seychelles			
Se 44558		dengue, type 2	

Table 49 continued

Virus referred to YARU for identification and study, 1982

Country of origin; strain	Source	Information from donor	YARU identification
Singapore			
Sing 16182	human serum	dengue, type 3	
South Africa			
Natal bat	wild bat	rabies related	Strain of Lagos bat
SA-Congo	ticks	Crimean-Congo HF virus	
SA 640	wild bat	rabies-related	rabies-related
SA 679	wild bat	rabies-related	rabies-related
SA 1248	wild bat	rabies-related	rabies-related
SA 1486	wild bat	rabies-related	rabies-related
Sri Lanka			
SL 228800	human serum	dengue, type 4	
Tahiti			
Ta 18	human serum	dengue, type 3	
Ta 79	human serum	dengue, type 4	
Thailand			
Thai Bkk 16803	human serum	dengue, type 2	
Thai 49080	human serum	dengue, type 3	
USA			
Ieri, TR8762		For confirmation as an orbivirus	
USA-PR	acute phase human serum	suspect dengue	dengue, type 4

Table 49 (continued)

Virus referred to YARU for identification and study, 1982

Country of origin; strain	Source	Information from donor	YARU identification
USA (continued)			
USA MS1-7			St. Louis encephalitis
USA R 19420			Colorado tick fever
USA S6 14-03			Colorado tick fever
USA 75V1843			Colorado tick fever
USA 75V3863			Colorado tick fever
USA 69V28			Colorado tick fever
Eyach (original)			Eyach
78V3531			VEE, subtype IF
Ag 80-663			VEE, type 6
TB4-1054			Bahia Grande
79V5816			
USA An 4090			
USA BFS 1703			western encephalitis
LT 1			
LT 2			
D5			
Lost H			Colorado tick fever
HLP			Colorado tick fever
Florio/p29			Colorado tick fever
Harris			Colorado tick fever
LH			Colorado tick fever
Suckling mouse cataract agent	tick		Spiroplasma
Murre H			Murre
USA 109214D	Ornithodoros occidentalis		Cascade
Texas 69			Sunday Canyon
Sapphire I			Ungrouped
Alk 71-1628			Kachemah virus, Sakhalin gp.
66394-B			
47156			Green Kure

Table 49 (continued)

Virus referred to YARU for identification and study, 1982

Country of origin; strain	Source	Information from donor	YARU identification
USA continued			
Aedes albopictus CTF			Colorado tick fever
5518			Colorado tick fever
66944B			Colorado tick fever
105365	Argas coolei		Six Gun City, Kemerovo gp.
105353	Ixodes uriae		Yaquina Head plus Sakhalin
105355	Ixodes uriae		new member, Uukuniemi gp.
109166B	Ixodes pacificus		
60121-62			Hughes group
63668-4			Soldado Rock, Hughes group
65660-59			Soldado Rock, Hughes group
66269-34a			
66484			Six Gun City, Kemerovo group
Garcia			Colorado tick fever
Alk bird blood			Kemerovo group
65821-3			Aves Island virus, Hughes gp.
113704-14	Ornithodoros con.		
109213D			Cascade virus
66270-19			Hughes group
57759-48			Hughes group
75V 12076	Culex pipiens		St. Louis encephalitis
TNM4-212	Culex pipiens		St. Louis encephalitis
Northway virus			new virus, Bunyamwera gp.
Naranjal virus			new virus
66-1550	Anopheles quadrimaculatus		Bunyamwera gp.
66-1850	Anopheles punctipennis		Bunyamwera gp.
66-2126	Anopheles quadrimaculatus		Bunyamwera gp.
66-3212	Coquilitidia perturbans		Bunyamwera gp.
75-1615	Anopheles punctipennis		Bunyamwera gp.
75-874	Anopheles punctipennis		Bunyamwera gp.

Table 49 (continued)

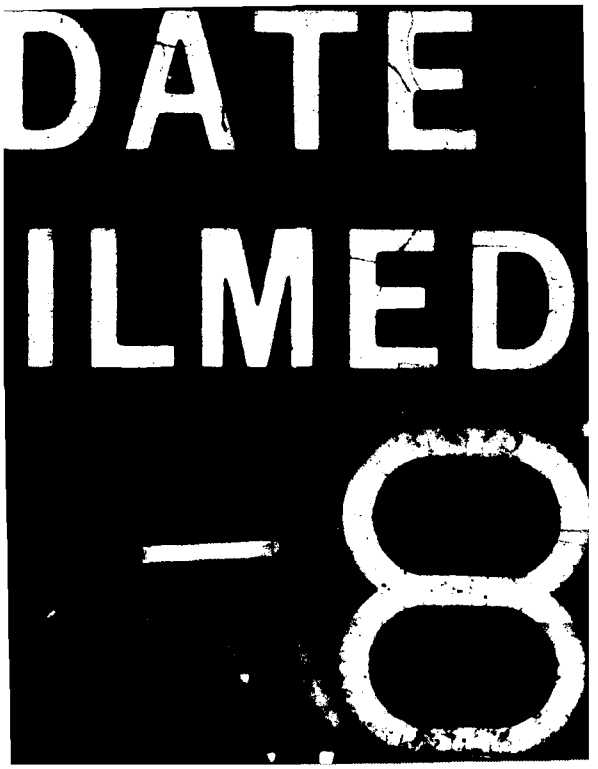
Virus referred to YARU for identification and study, 1982

Country of origin; strain	Source	Information from donor	YARU identification
USA continued			
78-1974	Aedes trivittatus	Bunyamwera gp.	
78-4582	Anopheles punctipennis	Bunyamwera gp.	
78-5874	Aedes trivittatus	Bunyamwera gp.	
78-5912	Anopheles quadrimaculatus	Bunyamwera gp.	
78-7286	Aedes trivittatus	Bunyamwera gp.	
78-7367	Aedes trivittatus	Bunyamwera gp.	
78-7402	Aedes trivittatus	Bunyamwera gp.	
78-7435	Aedes trivittatus	Bunyamwera gp.	
78-7437	Aedes trivittatus	Bunyamwera gp.	
78-7457	Aedes trivittatus	Bunyamwera gp.	

Table 50

Vertebrate and invertebrate cell lines distributed during 1982

Name of recipient	Location	Date sent	Cell line	Number	Size of flask
Dr. C.J. Peters	USAMRIID, Frederick, MD	12 March	Lutzomyia longipalpis	2	25 cm^2
Dr. M. Rebello	Rio de Janeiro, Brazil	19 March	Aedes albopictus C6/36	1	25 cm^2
Dr. O. Wood	NAMRU-3, Cairo	23 March	Aedes albopictus C6/36	1	25 cm^2
Dr. B.A. Vaxton	Auburn, AL	13 April	Aedes pseudoscutellaris	2	25 cm^2
			Aedes albopictus C6/36	2	25 cm^2
Dr. T.G. Ksiazek	Berkeley, CA	10 May	XTC-2 (toad)	1	25 cm^2
Dr. P. Hsieh	Cambridge, MA	17 May	Aedes pseudoscutellaris	1	25 cm^2
			Aedes albopictus C6/36	1	25 cm^2
Dr. J. Peleg	Biet-Dagan, Israel	30 July	Lutzomyia longipalpis	1	25 cm_2
			Aedes albopictus C6/36	1	25 cm^2
Dr. H. Artsob	Toronto, Canada	29 Sept.	Aedes albopictus C6/36	1	25 cm^2
Dr. I.P. Schneider	WRAIR, Washington, DC	22 Nov.	Lutzomyia longipalpis	1	25 cm^2
			Phlebotomus papatasi	1	25 cm^2
Dr. C. Rossi	Auburn, AL	22 Nov.	Sarcoma 180/TG	1	5 ml
Dr. C. House	USDA, Plum Island, NY	12 Dec.	Aedes albopictus C6/36	1	25 cm^2
			Dermacentor variabilis RML-15	3	25 cm^2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